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Global transmission network and driving mechanisms: An epidemiological and genomic study of bla OXA -mcr co-carrying strains

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Abstract

Purpose

The co-carriage of carbapenem-resistant *bla*$_{OXA}$ genes and colistin-resistant *mcr* genes in bacterial strains represents a critical threat to public health by undermining last-resort antibiotics. This study aims to systematically investigate the global prevalence, transmission dynamics, and genetic drivers of *bla*$_{OXA}$-*mcr* co-carrying strains to inform surveillance and intervention strategies.

Methods

We conducted a comprehensive analysis of 3,665 bacterial genomes from the NCBI database (1953–2024), identifying 284 *bla*$_{OXA}$-*mcr* co-carrying strains. Using bioinformatics tools, we characterized their spatiotemporal distribution, phylogenetic relationships, plasmid environments, and mobile genetic element (MGE) structures.

Results

The 284 strains were isolated from 30 countries, with Germany showing the highest detection rate (47.2%). The dominant genotype combination was *bla*$_{OXA-48}$–*mcr-10* (74.3%), primarily carried by Enterobacteriaceae. Phylogenetic analysis confirmed transcontinental and cross-host transmission events, including a Romania–UK clone differing by only one SNP. The species composition shifted from Acinetobacter baumannii to Enterobacteriaceae, and the host spectrum expanded to include pets and environmental samples after 2016. *bla*$_{OXA}$ genes were predominantly plasmid-borne (66.9%), mainly on IncL plasmids, while mcr genes were frequently located on IncHI2A plasmids. Notably, flanking MGE structures exhibited a trend toward simplification, potentially reducing fitness costs.

Conclusion

bla $_{OXA}$-*mcr* co-carrying strains have formed a complex global transmission network transcending national, species, and host barriers, driven by synergistic plasmid–clone co-evolution. These findings underscore the urgent need for proactive One Health surveillance and targeted interventions to mitigate the spread of these dual last-resort resistant pathogens.

Introduction

Antimicrobial resistance (AMR) has been listed by the World Health Organization (WHO) as one of the "top ten global health threats" and has become a priority issue in international public health governance. In 2019, an estimated 1.27 million deaths were directly attributable to bacterial infections caused by antimicrobial resistance[1]. The latest forecasts indicate that between 2025 and 2050, the average annual number of deaths directly attributable to AMR will reach 1.91 million, with an estimated cumulative 39.1 million deaths over this period[2]. In this critical context, clinical anti-infective therapy is increasingly constrained by the failure of conventional antibiotics, leading to a growing scarcity of “last-resort” treatment options.

Carbapenems have long been regarded as last-resort antibiotics for severe infections caused by ESBL-producing Enterobacterales[3]. However, global surveillance data from 2023 indicate that carbapenem resistance is escalating across multiple bacterial species, with rates reaching 54.3% for *Acinetobacter baumannii* and 16.7% for *Klebsiella pneumoniae*. Among these, the global spread of carbapenem-resistant Enterobacterales (CRE)—primarily driven by bacterial production of carbapenemases—has substantially compromised the clinical efficacy of these agents[1].

Carbapenem-hydrolyzing class D β-lactamases (CHDLs) are a subset of oxacillinases (OXA enzymes) and key members of the carbapenemase family. Since the first carbapenem-hydrolyzing OXA enzyme (OXA-23) was reported in Acinetobacter baumannii in 1993[4], this family has expanded to over 700 variants with confirmed carbapenem-hydrolyzing activity. Critically, unlike carbapenemase genes such as bla_{NDM} and bla_{KPC}—which universally encode carbapenemases—the OXA family is marked by heterogeneity: not all members possess carbapenemase activity, with significant functional divergence and host spectrum differences across its subfamilies.

Among these, the $bla_{OXA-23-like}$ subgroup exhibits the strongest carbapenem-hydrolyzing activity and has spread beyond Acinetobacter baumannii to Enterobacterales, albeit sporadically[5]. Although the overall detection rate in Enterobacterales remains low, this finding suggests that its host range is expanding. In contrast, $bla_{OXA-51-like}$ is a chromosome-borne intrinsic gene of *Acinetobacter baumannii*[6]. It exhibits extremely weak basal hydrolytic activity; however, upstream insertion of ISAba1 can upregulate its expression, leading to high-level resistance. This "conditional activation" characteristic makes it easily underestimated in routine detection[7, 8].

Unlike the two subgroups described above, members of the $bla_{OXA-48-like}$ family generally exhibit weak and variable carbapenem-hydrolyzing activity (OXA-181 > OXA-48 > OXA-232) [9], and some variants (e.g., OXA-163, OXA-405) have even lost this activity[10, 11]. Consequently, conventional phenotypic susceptibility testing is prone to yield “false-susceptible” results[12, 13], rendering $bla_{OXA-48-like}$-carrying strains a “hidden” reservoir of resistance that enables silent dissemination in hospitals and communities undetected, thereby constituting a significant clinical surveillance blind spot. Despite this detection challenge, OXA-48 and its derivatives (e.g., OXA-181, OXA-232) have achieved global dissemination via broad-host-range plasmids (e.g., IncL, IncX3), becoming a major carbapenem resistance mechanism in Enterobacterales[14, 15] and conferring multidrug-resistant (MDR) or extensively drug-resistant (XDR) phenotypes that complicate clinical treatment[16]. The features—functional divergence, surveillance blind spots, and efficient plasmid-mediated transmission—make bla_{OXA} monitoring particularly challenging and underscore the urgent need for systematic investigation.

As the efficacy of carbapenems continues to decline, clinicians have been compelled to resort to colistin—an agent previously withheld owing to its nephrotoxicity and neurotoxicity. However, the emergence of the plasmid-mediated colistin resistance gene *mcr-1* in 2015 fundamentally altered this landscape [17]. To date, more than ten *mcr* variants, including *mcr-10*, have been identified and are widely distributed among humans, livestock, pets, and environmental samples[18]. The use of colistin as an agricultural growth promoter[19], together with clinical antibiotic selective pressure, has driven the global dissemination of these resistance genes, threatening the clinical effectiveness of colistin.

Importantly, the co-carriage of carbapenemase-encoding and colistin resistance genes is increasingly observed, conferring dual resistance to both classes of "last-resort" antibiotics. In recent years, clinical and environmental isolates co-carrying carbapenemase-encoding genes (e.g., bla_{OXA-48}) and colistin resistance genes (e.g., *mcr-1*) have been repeatedly reported [20]. However, previous studies have primarily examined the independent dissemination of

bla$_{OXA}$ or *mcr* [21–23], leaving the global prevalence, transmission dynamics, and underlying genetic drivers of strains co-carrying carbapenem-hydrolyzing *bla*$_{OXA}$ genes and the colistin resistance gene *mcr* (*bla*$_{OXA}$-*mcr* co-carrying strains) largely unexplored: first, the host spectrum and resistance phenotypes vary significantly across *bla*$_{OXA}$ subfamilies, creating epidemiological blind spots; their actual distribution and contribution in *bla*$_{OXA}$-*mcr* co-carrying strains remain unclear; second, the transmission ecology is highly complex; the human gut, animal hosts, and the environment have been identified as "hotspots" for resistance gene dissemination[24], but whether and how *bla*$_{OXA}$-*mcr* co-carrying strains navigate these One Health interfaces for cross-species and cross-regional transmission remains unknown; and third, the underlying genetic drivers have yet to be elucidated; do *bla*$_{OXA}$ and *mcr* genes preferentially co-localize on the same plasmid to form a "super-vector," or do they stably coexist and spread on distinct genetic carriers, and what adaptive evolutionary changes have occurred in the flanking mobile genetic elements (MGEs) during their prolonged dissemination?

To address these questions, this study conducted a systematic mining and analysis of global public genomic databases to characterize the global prevalence, transmission network, and genetic basis of *bla*$_{OXA}$-*mcr* co-carrying strains at the macro level. Specifically, this study aims to: (1) characterize the global spatiotemporal distribution, predominant host species, and core resistance genotype combinations of *bla*$_{OXA}$-*mcr* co-carrying strains; (2) reveal their geographic distribution patterns, species-region associations, and transcontinental and cross-host transmission events through phylogenetic genomic analysis; and (3) dissect the genetic environment of *bla*$_{OXA}$ and *mcr* genes—including their chromosomal or plasmid localization, plasmid vector types, and flanking MGE structures—to elucidate the molecular mechanisms underlying their co-dissemination and co-evolution. By integrating epidemiological and bioinformatics approaches, this study aims to provide essential evidence and data support for establishing a global active surveillance network targeting such extremely drug-resistant bacteria, assessing their transmission risks, and formulating One Health-based intervention strategies, thereby answering these questions to better predict epidemiological trends and design targeted interventions.

Results

1. Global prevalence and diversity of *bla*$_{OXA}$-*mcr* co-carrying strains

To investigate the global prevalence and distribution of strains co-carrying carbapenem-resistant *bla*$_{OXA}$ genes and colistin-resistant *mcr* genes (here in after referred to as *bla*$_{OXA}$-*mcr* co-carrying strains), we systematically searched and screened relevant genomic data from the NCBI database (Supplementary File 1). A total of 3,665 bacterial genomes, spanning the period from 1953 to 2024, were included. Based on the functional classification of the Beta-Lactamase Database (BLDB) (as of January 2026, BLDB has catalogued 1,358 OXA enzymes across 90 subfamilies, of which 722 possess confirmed carbapenem-hydrolyzing activity, primarily distributed among the OXA-23-like, OXA-48-like, and OXA-51-like subfamilies; see Supplementary File 2 and Table 1), BLAST sequence alignment was performed on the 3,665 strains. Ultimately, 284 *bla*$_{OXA}$- *mcr* co-carrying strains were identified, constituting the core dataset for subsequent analyses.

Table 1
Functional classification of OXA enzymes

Function	subfamily	OXA
Carbapenemase-Resistant (722)	OXA-10-like (1)	OXA-233;
	OXA-23-like(48)	OXA-23, OXA-27, OXA-73…et;
	OXA-24-like (15)	OXA-24, OXA-25, OXA-26…et;
	OXA-48-like (16)	OXA-48, OXA-54, OXA-162, OXA-181…et;
	OXA-51-like (352)	OXA-51, OXA-64, OXA-65…et;
	OXA-213-like (72)	OXA-213, OXA-267, OXA-268…et;
	Other family (248)	OXA-55-like, OXA-58-like, OXA-60-like, OXA-62-like, OXA-134-like, OXA-143-like, OXA-153-like, OXA-154-like…et;
No-Carbapenemase-resistant (87)	OXA-1-like (5)	OXA-1, OXA-31, OXA-796...et;
	OXA-2-like (8)	OXA-2, OXA-15, OXA-53...et;
	OXA-10-like (15)	OXA-10, OXA-11, OXA-14...et;
	OXA-48-like (2)	OXA-163, OXA-405;
	OXA-50-like (54)	OXA-1244, OXA-1245, OXA-1246...et;
	Other family (3)	OXA-20-like, OXA-22-like, OXA-85-like,
Unknown (549)	OXA-1-like (16)	OXA-224, OXA-320, OXA-392...et;
	OXA-2-like (25)	OXA-3, OXA-21, OXA-415...et;
	OXA-12-like (13)	OXA-12, OXA-724, OXA-725...et;
	OXA-23-like (3)	OXA-105, OXA-481, OXA-1095;
	OXA-48-like (57)	OXA-252, OXA-370, OXA-416...et;
	OXA-51-like (45)	OXA-852, OXA-853, OXA-854...et;
	Other family (390)	OXA-5-like, OXA-9-like, OXA-42-like, OXA-46-like, OXA-50-like...et;

These 284 strains were isolated between 2008 and 2024, originating from 30 countries and regions worldwide. The strains showed a highly uneven geographic distribution, with Germany accounting for the highest proportion (47.2%,

134/284), followed by Spain (10.6%, 30/284), the United Kingdom (5.6%, 16/284), Singapore (4.2%, 12/284), and China (3.9%, 11/284) (Fig. 1A). Among all *bla*$_{OXA}$-*mcr* co-carrying strains, 276 belonged to Enterobacterales, while the remaining eight were *Acinetobacter baumannii* (family Moraxellaceae) (Fig. 1B). The predominant species were *Citrobacter freundii*, *E.coli and Shigella*, *Klebsiella pneumoniae*, and *Enterobacter hormaechei*.

At the resistance genotype level, 23 distinct *bla*$_{OXA}$-*mcr* combination patterns were identified (Fig. 1C). The *bla*$_{OXA-48}$-*mcr-10* combination was overwhelmingly dominant (74.3%, 211/284), followed by *bla*$_{OXA-48}$-*mcr-1* (7.4%, 21/284) and *bla*$_{OXA-181}$-*mcr-1* (2.8%, 8/284). Further analysis revealed distinct distribution preferences of these combinations at both country and genus levels (Fig. 1A). For instance, the dominant *bla*$_{OXA-48}$-*mcr-10* combination was widely prevalent across multiple major source countries, including Germany, Spain, the United Kingdom, and China. In contrast, the *bla*$_{OXA-232}$-*mcr-1* combination was exclusively identified in Asian countries such as Singapore and Thailand, suggesting the presence of regionally disseminated clones. At the genus level, combinations involving *bla*$_{OXA-65}$, *bla*$_{OXA-67}$, and *bla*$_{OXA-69}$ (belonging to the *bla*$_{OXA-51}$ family) were restricted to *Acinetobacter baumannii*, whereas all other *bla*$_{OXA}$-*mcr* combinations were widely distributed across various Enterobacterales species. Notably, *Klebsiella pneumoniae* and *E.coli* and *Shigella* strains harbored the most diverse range of genotype combinations (Fig. 1B), suggesting that these species may possess enhanced adaptability or a greater capacity for horizontal gene transfer in the acquisition and reassortment of resistance genes.
Further resistome analysis revealed that *bla*$_{OXA}$-*mcr* co-carrying strains generally exhibited a broad multidrug resistance background. A co-occurrence network analysis of resistance genes (Fig. 1D) showed that all resistance genes were tightly interconnected, suggesting a high degree of co-existence among these resistance determinants in the bacterial population. Notably, *bla*$_{OXA-48}$ was located at the center of the network, consistent with its high detection frequency. The central region of the network was also enriched with numerous other resistance genes, revealing a concentrated distribution of multidrug resistance mechanisms within the *bla*$_{OXA}$-*mcr* co-carrying strain population. Further analysis revealed that this population also frequently carried other carbapenemase genes, including *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, and *bla*$_{VIM}$, and co-harbored a wide array of resistance genes conferring resistance to aminoglycosides, fluoroquinolones, sulfonamides, trimethoprim, and tetracyclines (Table 2). The resistance gene profile was characterized by high diversity and abundance.

Table 2
Occurrence frequencies of all antibiotic resistance genes in *bla*$_{OXA}$–*mcr* co-carrying strains

Category	Gene-Count	Total_Occurrences
Beta-lactam	83	510
Aminoglycoside	41	972
Carbapenem	28	397
Quinolone	27	303
Sulfonamide	22	497
Polymyxin	15	341
Chloramphenicol	10	322
Tetracycline	9	179
Other	77	1005

2. Spatiotemporal transmission dynamics and host spectrum evolution of *bla*$_{OXA}$-*mcr* co-carrying strains

Spatiotemporal distribution

To elucidate the spatiotemporal transmission and host evolution of *bla*$_{OXA}$-*mcr* co-carrying strains, we analyzed the temporal, geographic, and host distribution of the core dataset. The earliest strain was an *Acinetobacter baumannii* isolate (SAMN10411605) from Brazil in 2008, co-carrying *bla*$_{OXA-65}$ last-resort, *bla*$_{OXA-23}$ last-resort, and last-resort *mcr-4* last-resort. Since then, the geographic distribution has expanded continuously, with detection countries increasing annually, including Germany, China, and others. Although the spread has slowed since 2019, new countries continue to report these strains each year, indicating a persistent risk of cross-border transmission (Figs. 2A, 2D).

Temporal distribution analysis (Fig. 2A) revealed that the global number of reported *bla*$_{OXA}$-*mcr* co-carrying strains increased steadily between 2013 and 2016, peaked in 2019, and then declined sharply. The epidemic patterns varied markedly across countries. Germany experienced a sustained circulation between 2018 and 2021. In contrast, the United Kingdom showed a concentrated reporting period only from 2014 to 2016, suggesting possible local surveillance or intervention efforts during that time. Spain, however, maintained a relatively stable and consistent detection level throughout the study period, indicating potential endemic transmission.

Species composition evolution

The species composition of *bla*$_{OXA}$-*mcr* co-carrying strains exhibited marked temporal evolution. Between 2008 and 2016, species diversity increased rapidly, reflecting the rapid spread of these resistant strains across different bacterial species. During this period, the composition expanded from a single species—*Acinetobacter baumannii*—to a wide range of Enterobacterales. After 2016, the increase in diversity slowed, and all newly emerging species belonged to Enterobacterales. By 2019, the diversity had stabilized, forming a complex population structure comprising multiple species, including Klebsiella pneumoniae, *E.coli* and *Shigella*, and others (Figs. 2B, 2D). This suggests that the dissemination of this resistance gene combination has become relatively concentrated within Enterobacterales.

Meanwhile, the number of strains increased continuously from 2017 onward, peaked in 2019, and then declined sharply, returning to pre-epidemic levels by 2022. During this period, the dominant species underwent three shifts: in 2019, *Citrobacter freundii* was overwhelmingly dominant; from 2020 to 2021, *Enterobacter roggenkampii* became the dominant species; and in 2022, *Enterobacter hormaechei* replaced it. Notably, *Citrobacter freundii* was the most frequently detected species throughout the study period (detected continuously from 2012 to 2022), while *Enterobacter hormaechei* and *Klebsiella pneumoniae* had been detected continuously since 2010 (Figs. 2B, 2D). This evolutionary trajectory suggests that the *bla*$_{OXA}$-*mcr* gene combination has not only spread within the same species but has also successfully transferred and established itself across different species, thereby forming dominant epidemic clones within Enterobacterales. This may have accelerated the dissemination of multidrug-resistant phenotypes among key pathogens such as Enterobacterales.

Niche expansion and transmission network

Host source analysis further revealed an expanding ecological distribution trend (Fig. 2C). Although human clinical samples predominated throughout the study period, *bla*$_{OXA}$-*mcr* co-carrying strains have been successively detected

in companion animals (cats, dogs), livestock (pigs), and environmental samples (water, soil) since 2016. From a dynamic perspective, the host spectrum showed a clear expansion trend between 2016 and 2021 (Fig. 2D), gradually extending from a primary focus on humans to encompass livestock, environmental sources, and other hosts, forming a multi-host coexistence transmission pattern. After 2021, host spectrum expansion stabilized, with no significant addition of new hosts. This shift indicates that the ecological niche of these strains has progressively permeated from a predominantly human-restricted range to the human–animal–environment interface, suggesting an active risk of cross-species transmission chains and the formation of environmental reservoirs.

Collectively, these findings demonstrate that *bla*$_{OXA}$-*mcr* co-carrying strains have formed a complex transmission network that transcends national, species, and host barriers. Their ecological adaptability and transmission potential continue to strengthen, highlighting the severe and evolving public health threat they pose within the One Health framework.

3. Phylogenetic and evolutionary relationships of *bla*$_{OXA}$-*mcr* co-carrying strains

To elucidate the evolutionary relationships and transmission patterns of *bla*$_{OXA}$-*mcr* co-carrying strains, we constructed a phylogenetic tree based on the bacterial core gene set (bac120) including all 284 strains (Fig. 3). Phylogenetic analysis integrated with geographic information revealed complex transmission dynamics. Strains from Germany were widely distributed across multiple distinct evolutionary clades, primarily among Enterobacterales species such as *Citrobacter freundii*, *E. coli* and *Shigella*, *Enterobacter roggenkampi*i, and *Enterobacter asburiae*. Of these, 97% carried the *bla*$_{OXA-48}$-*mcr-10* combination. Germany had continuous strain isolation records throughout the study period, suggesting that the country may have played a key role as a hub in the transmission and evolution of *bla*$_{OXA}$-*mcr* co-carrying strains, experiencing multiple independent introductions and local transmission events rather than a single-source diffusion. In contrast, although data from the United Kingdom were relatively limited, the continuous isolation records between 2013 and 2016 also suggest that this resistance determinant established a short-lived transmission chain locally.

The phylogenetic structure also revealed certain species–region association patterns. For example, *Citrobacter freundii*, the most prevalent species, was mainly concentrated in European countries such as Germany, Ireland, the United Kingdom, and Spain, whereas *Kluyvera intermedia* was exclusively identified in Spanish samples. A particularly critical finding was the identification of 12 pairs of strains that, despite being isolated from different countries (e.g., Germany, the United Kingdom, Thailand, and China) and different years (2008–2021), were located within the same clade and were each other's nearest neighbors in the phylogenetic tree (Fig. 3). This observation suggests potential cross-regional and cross-temporal transmission events involving these strain pairs. To test this hypothesis, we further assessed the genetic distances of these strain pairs using whole-genome SNP analysis and average nucleotide identity (ANI) calculations. The results showed that the SNP differences among the 12 pairs ranged from 0 to 21, and ANI values ranged from 92.82% to 99.9984% (Table 3). Among them, pair1 (a 2016 Romanian isolate vs. a 2020 UK isolate) exhibited the smallest genetic distance, with only one SNP difference and an ANI of 99.9984%, confirming a cross-temporal and cross-border transmission clone. Both strains were *E. coli and Shigella* co-carrying *bla*$_{OXA-162}$ and *mcr-1*, isolated from poultry (Romania, 2016) and an environmental sample (UK, 2020), respectively, suggesting a potential transmission pathway between livestock and the environment.

Host association analysis provided further clues for understanding local transmission chains. For instance, all canine-origin strains were isolated from Switzerland and clustered within the same clade as human-origin strains from the same country. These canine and human strains shared highly consistent resistance gene profiles,

predominantly featuring the *bla*$_{OXA-48}$-*mcr-10* combination, strongly suggesting cross-species transmission of resistant bacteria between humans and pets through close contact in specific community or household settings.

Collectively, the phylogenetic genomic analysis indicates that the global transmission network of *bla*$_{OXA}$-*mcr* co-carrying strains is complex, characterized by multiple origins and multiple pathways. Their dissemination is simultaneously influenced by geographic distance, host species, and ecological niches. However, frequent cross-regional gene exchange, successful cross-host adaptation, and the long-term stability of clonal lineages have collectively driven the global spread and persistence of this resistance phenotype. These findings provide critical genomic insights for understanding the transmission networks of extremely drug-resistant bacteria from an evolutionary perspective and for developing targeted surveillance and intervention strategies.

Table 3
SNP and ANI analysis of the 12 strain groups

Pair	Strain A	Country A	YearA	Strain B	Country B	Year B	ANI(%)	SNP
pair1	GCA_015123715.1	UK	2020	GCA_019003245.1	Romania	2016	99.9984	1
pair2	GCA_022116895.1	UK	2016	GCA_030414295.1	Belgium	2021	99.9125	0
pair3	GCA_022158145.1	UK	2016	GCA_043828705.1	Germany	2020	99.8363	0
pair4	GCA_018446915.1	Germany	2019	GCA_905330765.2	Spain	2018	99.7584	0
pair5	GCA_024736545.1	Spain	2019	GCA_040697285.1	France	2022	99.7097	0
pair6	GCA_014117525.2	Thailand	2017	GCA_048019365.1	France	2017	99.6767	0
pair7	GCA_013201195.1	China	2016	GCA_022154035.1	UK	2016	99.6174	0
pair8	GCA_024579815.1	China	2022	GCA_028561825.1	Spain	2021	99.5712	21
pair9	GCA_025752295.1	Germany	2019	GCA_041644895.1	Thailand	2022	99.1009	0
pair10	GCA_022146185.1	UK	2015	GCA_964331145.1	Ireland	2017	98.4017	0
pair11	GCA_003812485.1	Brazil	2008	GCA_003994455.1	China	2018	97.5713	0
pair12	GCA_021545035.1	UK	2014	GCA_022543595.1	Germany	2019	92.8161	0

4. Plasmid environment of *bla*$_{OXA}$ and *mcr* genes

To elucidate the dissemination mechanisms of key resistance genes in *bla*$_{OXA}$-*mcr* co-carrying strains, we systematically analyzed the genetic localization, plasmid vectors, and flanking environments of *bla*$_{OXA}$ and *mcr* genes. Based on whole-genome data, we distinguished chromosomal from plasmid sequences using bioinformatics tools and systematically identified plasmid replicon types (Fig. 4A). The results showed that *bla*$_{OXA}$ genes were predominantly located on plasmids (66.9%, 190/284), with plasmid types identified in 25.0% (71/284) of strains; only 4.2% (12/284) carried these genes on chromosomes. *mcr* genes were also mainly plasmid-borne (43.3%, 123/284), with plasmid types identified in 23.2% (66/284) of strains; chromosomal localization accounted for 20.8% (59/284). Notably, 58 strains carried both *bla*$_{OXA}$ and *mcr* genes on identifiable plasmids.

Among strains with identifiable plasmid types, *bla*$_{OXA}$ genes were primarily associated with IncL, IncX3, and ColKP3 plasmids, while *mcr* genes were mainly located on IncX4, IncI2(Delta)/IncI2, and IncHI2A plasmids. A total of 18

distinct "*bla*$_{OXA}$ plasmid–*mcr* plasmid" combinations were identified, with IncL–IncHI2A being the most common (37/58), suggesting that these two plasmid types may synergistically mediate the co-dissemination of dual-resistance phenotypes. Specific associations between plasmid types and resistance gene subtypes were also observed. *bla*$_{OXA-48}$ was primarily located on IncL plasmids, whereas *bla*$_{OXA-181}$ and *bla*$_{OXA-232}$ were frequently associated with ColKP3 plasmids. *mcr-1* was distributed across multiple plasmid types, including IncX4, IncX1, IncI2(Delta)/IncI2, and IncHI2A, while *mcr-10* was highly enriched on IncHI2A plasmids.

Temporal trend analysis (Fig. 4B) revealed that annual fluctuations in *bla*$_{OXA}$ genes were primarily driven by plasmid-borne *bla*$_{OXA}$, whereas chromosomally located *bla*$_{OXA}$ remained relatively stable. In contrast, the fluctuation patterns of *mcr* genes were more complex. Before 2013, *mcr* genes were predominantly chromosome-borne; however, since the second half of 2013, plasmid-borne *mcr* has gradually become dominant. Nevertheless, chromosomally located *mcr* maintained a certain level of abundance with notable interannual fluctuations, indicating that changes in total *mcr* gene abundance are influenced by both plasmid-borne and chromosome-borne *mcr*, which showed relatively similar fluctuation trends. These dynamic patterns reveal differences in the dissemination mechanisms of the two resistance gene classes: *bla*$_{OXA}$ genes rely primarily on plasmid-mediated horizontal transfer, whereas *mcr* genes may disseminate through both vertical and horizontal pathways. Notably, although the fluctuations of plasmid-borne *mcr* and plasmid-borne *bla*$_{OXA}$ were not entirely consistent, chromosome-borne *mcr* showed temporal patterns similar to those of plasmid-borne *bla*$_{OXA}$. This observation suggests that horizontal transfer of *bla*$_{OXA}$ may occur in strains carrying chromosome-borne *mcr*, forming a synergistic evolutionary model of "vertical localization–horizontal dissemination" at the population level.
The host range of plasmids critically influences their transmission efficiency (Fig. 4C). Narrow-host-range plasmids, such as IncFII and its variants, are generally restricted to specific hosts like *Escherichia coli*. Col-type plasmids, although mainly found in *Klebsiella pneumoniae*, also occur in other Enterobacterales such as *E. coli* and *Shigella*, *Citrobacter freundii*. In contrast, broad-host-range plasmids (e.g., IncL, IncHI2A, IncX3, IncX4) can transfer among multiple Enterobacterales species, greatly facilitating cross-species dissemination of resistance genes. All plasmids carrying *bla*$_{OXA}$ or *mcr* identified in this study belonged to Enterobacterales-associated plasmid types, with host ranges relatively conserved and restricted to Enterobacterales. Geographic distribution analysis (Fig. 4D) showed that IncL and IncHI2A plasmids dominated the spread of *bla*$_{OXA}$ genes across multiple countries (e.g., China, Croatia, France, Germany), whereas plasmids such as IncFII(pCoo) were confined to specific regions (e.g., Singapore).

Furthermore, in two strains, *bla*$_{OXA}$ and *mcr* genes co-localized on the same plasmid (IncFII(pECLA)), which typically exists as a multi-replicon fusion plasmid formed by the integration of resistance or virulence genes carried by transposons or insertion sequences into a conjugative IncFII plasmid backbone. Such plasmids possess high conjugative transfer capacity and impose low fitness costs on the host, features that may further accelerate the co-dissemination of dual-resistance genes.

5. Structural diversity of mobile genetic elements flanking *bla*$_{OXA}$ genes

To dissect the local genetic context of *bla*$_{OXA}$ genes, we systematically annotated all strains using Bakta and analyzed the flanking sequences of 349 *bla*$_{OXA}$ genes. The results showed that none of the detected *bla*$_{OXA}$ genes were located within known classical transposon structures (e.g., Tn1999, Tn2006) [22]; however, their flanking regions exhibited family-specific and regular structural features. Sequence structure analysis (Fig. 5A) revealed that among the dominant *bla*$_{OXA-48-like}$ genes (80.2%, 280/349), the flanking structures displayed distinct hierarchical characteristics: 54.6% (153/280) presented the simplest " *bla*$_{OXA-48}$–dmlA " unit, while 15.0% had an RNA-OUT

regulatory element integrated upstream of this unit. *bla*$_{OXA-162}$, belonging to the same family, contained an IS10A transposase-encoding sequence inserted at both the upstream and downstream ends based on the above structure. Additionally, elements such as IS10A, ISKpn19, QnrS1, and IS2_OrfA (frequency ≥ 2%) were frequently detected flanking *bla*$_{OXA-48-like}$ genes. In contrast, *bla*$_{OXA-23}$ family genes (4/349) exhibited a highly conserved structure, consisting solely of the "*bla*$_{OXA-23}$–ATP-binding protein" unit. The flanking regions of *bla*$_{OXA-51}$ family genes displayed even greater diversity, containing not only the FxsA antitoxin gene but also various functional elements such as acetyltransferases, oriC, and RuvC, reflecting the complexity accumulated during long-term evolution as chromosome-borne intrinsic genes.

These results indicate that the flanking structures of *bla*$_{OXA}$ genes exhibit marked family specificity, with complexity increasing progressively from the single conserved unit of *bla*$_{OXA-23-like}$, through the moderate hierarchical structures of *bla*$_{OXA-48-like}$, to the highly diverse elements of *bla*$_{OXA-51-like}$. Such structural differences may correlate with the evolutionary origins and transmission modes of each family. The minimalist structure of *bla*$_{OXA-48-like}$ genes may facilitate their rapid dissemination via plasmid-mediated horizontal transfer, whereas the high diversity of *bla*$_{OXA-51-like}$ flanking regions reflects continuous recombination and adaptation during their long-term evolution as chromosome-borne intrinsic genes.
To systematically assess the variation patterns of flanking structures, we constructed a heatmap of flanking genes (Fig. 5B). The results showed significant sequence diversity at key positions both upstream (U1–U5) and downstream (D1–D7), with diversity increasing progressively from the core gene outward. This suggests that these flanking regions may be hotspots for genetic recombination and element insertion, and that their diversity likely influences the genetic plasticity and transmission fitness of *bla*$_{OXA}$ genes.

In summary, this study reveals the dissemination mechanisms of *bla*$_{OXA}$-mcr co-carrying strains at two levels: vectors and drivers. On one hand, broad-host-range plasmids (particularly IncL and IncHI2A) serve as primary vectors, providing "vehicles" for cross-species and cross-regional spread of these genes. On the other hand, the abundant mobile genetic elements flanking *bla*$_{OXA}$ genes and their gradient diversity provide sustained genetic drivers for gene capture and integration.

Discussion

The emergence of *bla*$_{OXA}$*-mcr* co-carrying strains marks a high-risk stage in bacterial antimicrobial resistance. These two genes target the two clinical “last-resort” antibiotic classes—carbapenems and colistin—respectively[25]. Their co-carriage converges independent resistance risk pathways into a single bacterium, leaving infected patients with no effective therapeutic options. This study found that *bla*$_{OXA}$*-mcr* co-carrying strains have been detected in 30 countries worldwide, forming a complex transmission network that transcends national, species, and host barriers. However, their geographic distribution is markedly uneven; the high detection rate in Germany may reflect intensive active surveillance, whereas the true prevalence in low- and middle-income countries is likely underestimated. Their dissemination is jointly driven by broad-host-range plasmids (e.g., IncL, IncHI2A), highly transmissible clones, and cross-species circulation at the human–animal–environment interface. Furthermore, the synergistic model of “vertical anchoring–horizontal acquisition” formed by chromosome-borne *mcr* genes and plasmid-borne *bla*$_{OXA}$ genes, together with the structural simplification of *bla*$_{OXA}$ flanking regions, confers strong genomic plasticity and transmission advantages upon these strains. These findings elucidate the global prevalence and transmission mechanisms of dual last-resort resistant strains, providing a scientific basis for developing surveillance and intervention strategies within the One Health framework.

1. Global prevalence of - co-carrying strains: Uneven distribution and true threat under surveillance bias

1. Global prevalence of *bla*$_{OXA}$-*mcr* co-carrying strains: Uneven distribution and true threat under surveillance bias

This study reveals that *bla*$_{OXA}$-*mcr* co-carrying strains have formed a global reservoir of resistance genes, with a markedly skewed geographic distribution. Despite a low overall detection rate, these strains have been reported in 30 countries (Fig. 1A), with significant clustering in Europe (Germany, Spain, and the UK). This pattern is not random but reflects the complex interplay between ecological boundaries and anthropogenic drivers in the global spread of multidrug-resistant genes within the One Health framework, consistent with previous reports[26].

However, when interpreting the above geographic distribution patterns, the potential impact of surveillance bias must be carefully considered. In this study, Germany reported the highest proportion of strains (Fig. 1A) with continuous isolation records (Fig. 2A). This phenomenon may be attributed to two factors. On one hand, it may reflect Germany's substantial investment in resistance surveillance systems, such as a large-scale active screening project targeting hospital wastewater in 2019[27], which identified numerous *bla*$_{OXA}$-*mcr*-carrying *Citrobacter freundii* strains in a single campaign, directly contributing to this species being the predominant one in our study (Figs. 1C, 2B). This systematic surveillance capacity objectively increased the detection probability of such strains. On the other hand, Germany exhibited non-episodic, relatively continuous strain distribution over time(Fig. 2A), and phylogenetic analysis further revealed that German strains were widely distributed across multiple distinct evolutionary clades (Fig. 3), suggesting that even after accounting for surveillance bias, sustained transmission and local evolution of *bla*$_{OXA}$-*mcr* co-carrying strains may still exist in Germany. In contrast, the United Kingdom showed a concentrated reporting period between 2014 and 2016 followed by a rapid decline (Fig. 2A), a temporal pattern that may be associated with intensified local surveillance or effective intervention measures during that period.

By comparison, data from many low- and middle-income countries, particularly regions known to be endemic for OXA-48 such as the Middle East and North Africa[28], may be severely under-represented. For example, a recent study in Algeria reported that *bla*$_{OXA\text{-}48\text{-}like}$ genes were the dominant carbapenemase genotype (82.12%) among carbapenem-resistant Enterobacterales, yet no *mcr* genes were detected in the relevant strains[29]. Given the potential lack of systematic surveillance networks or delayed data-sharing mechanisms in these regions, the true prevalence of *bla*$_{OXA}$-*mcr* co-carrying strains may be underestimated in this study. Global metagenomic studies have also indicated that the distribution of ARGs is primarily influenced by underlying host genera and biome types (e.g., human gut, wastewater) rather than by national borders[26].

Thus, systematic differences in surveillance capacity, transmission dynamics, intervention efficiency, and uneven coverage of global surveillance networks have collectively shaped the current skewed geographic distribution of *bla*$_{OXA}$-*mcr* co-carrying strains. Therefore, the high detection rate in Germany may reflect its active surveillance system more than indicating an absolute epicenter of dissemination, whereas data-deficient regions may harbor unrecognized transmission hotspots.

1. **Spatiotemporal expansion and niche extension: Transmission shift from a single host to the One Health network**

The transmission dynamics of *bla*$_{OXA}$-*mcr* co-carrying strains exhibited marked temporal fluctuations and regional heterogeneity. Globally, reported cases rose steadily from 2013, peaked in 2019, and then declined sharply (Fig. 2A).

This timeline coincides with the independent global dissemination waves of *bla*$_{OXA-48}$ (particularly IncL plasmid-mediated) and *mcr* genes (especially *mcr-1* and *mcr-10*)[22, 30], suggesting a temporal association between the prevalence of dual-resistant strains and the independent spread of the two resistance gene classes. Notably, country-specific temporal patterns varied considerably: Germany exhibited continuous detection from 2010 to 2022, with strains distributed across multiple phylogenetic clades (Fig. 3), suggesting multiple independent introductions and local transmission events. In contrast, the United Kingdom showed a concentrated reporting period between 2014 and 2016 followed by a rapid decline. These differences imply that, beyond the intrinsic transmissibility of bacteria and plasmids, local infection control measures and surveillance system sensitivity play equally important roles in determining whether resistance phenotypes become persistently established[31].

The species composition evolution further revealed selective characteristics of transmission. This study found that the species origin of *bla*$_{OXA}$-*mcr* co-carrying strains expanded from an initial single species *Acinetobacter baumannii* to Enterobacterales (Figs. 2B, 2D). This host shift is not incidental: Enterobacterales are widely distributed across the human–animal–environment interface[32] and their close contact with humans, animals, and the environment provides a natural channel for cross-interface dissemination of resistance genes. The dominant species underwent three shifts between 2019 and 2022, suggesting that the resistance gene combination is not fixed within a single clone but rather undergoes continuous diffusion within Enterobacterales.

Niche expansion is key to understanding their transmission potential. Before 2016, *bla*$_{OXA}$-*mcr* co-carrying strains were almost exclusively found in human samples; thereafter, their host spectrum expanded to include animals such as cats and dogs, as well as environmental samples (Fig. 2C). The timing of the emergence of these pet samples coincides with the detection of *mcr* genes in companion animals globally[33]. All canine-origin strains in this study originated from Switzerland and were phylogenetically highly homologous to human strains (Fig. 3), suggesting potential cross-species transmission of resistance genes between pets and humans. This finding echoes observations by Lei et al. in companion animals in Beijing, where the *mcr-1* positivity rate was 8.7% (49/566), and isolates from pet owners and their companion animals shared identical PFGE band patterns[34], further supporting the view that companion animals can serve as reservoirs of resistant bacteria and transmit them to humans through close contact. This reveals the limitations of traditional hospital-based infection control measures, which cannot easily reach household and community settings.

Transcontinental and cross-host clonal transmission events further highlight the ecological adaptability of these strains. In this study, a pair of *E. coli* and *Shigella* strains—one from poultry in Romania and the other from an environmental sample in the United Kingdom—formed each other's nearest neighbors in the phylogenetic tree (Fig. 3), differing by only one SNP, with an ANI of 99.9984% (Table 3), demonstrating high genomic stability over a four-year period. Given the typical bacterial evolutionary rate (approximately 1×10^{-7} SNPs/site/year), a four-year separation would normally be expected to accumulate dozens of SNPs[35]. The single nucleotide difference observed in this study suggests that this clone may have experienced evolutionary stasis or strong purifying selection during transmission. This clone successfully crossed geographic (Romania–UK) and host (poultry–environment) barriers, indicating broad ecological adaptability and posing a potential public health risk.

In summary, *bla*$_{OXA}$-*mcr* co-carrying strains have evolved from early sporadic human clinical cases into a complex transmission network transcending national, species, and host barriers. Their dissemination is jointly driven by the ecological niche advantages of Enterobacterales, community transmission chains mediated by companion animals, and cross-regional spread of highly stable clones. Future prevention and control strategies should extend

surveillance boundaries from healthcare facilities to households, communities, and ecological environments within the One Health framework.

3. Plasmid vectors and high-risk clones: Vehicles driving global dissemination

The global spread of *bla*$_{OXA}$-*mcr* co-carrying strains depends on the synergistic action of plasmid vectors and high-risk clones. In this study, the *bla*$_{OXA-48}$-*mcr-10* combination accounted for 74.3% of all strains, making it the absolutely dominant genotype (Fig. 1B). Among these, *bla*$_{OXA-48}$ was frequently located on broad-host-range plasmids such as IncL, while mcr-10 was highly associated with IncHI2A broad-host-range plasmids (Fig. 4A). IncL and IncHI2A plasmids possess high conjugative transfer efficiency and broad host ranges, making them key drivers of rapid dissemination within Enterobacterales [28, 36] and providing ideal molecular vehicles for the co-dissemination of both resistance gene classes. Previous reports indicated that *mcr-10* is highly inclined toward IncF-type plasmids; however, the diversity in plasmid types associated with *mcr-10* observed in this study suggests a possible link to its ability to adapt to different host environments.

The host range of plasmids directly determines the dissemination boundaries of resistance genes. All plasmids carrying *bla*$_{OXA}$ genes identified in this study belonged to Enterobacterales-associated plasmid types (Fig. 4A), with their hosts restricted to Enterobacterales. This characteristic explains the evolutionary pattern of species distribution among *bla*$_{OXA}$-*mcr* co-carrying strains. Although the first such strain was an *Acinetobacter baumannii* isolate from Brazil in 2008, this species has since appeared only sporadically (Fig. 1C). The reason lies in the fact that *bla*$_{OXA}$ genes in *A. baumannii* belong to the *bla*$_{OXA-51}$ family, which are chromosome-borne intrinsic genes of this species[6], whereas Enterobacterales-associated plasmids (e.g., IncL, IncHI2A) are poorly maintained in *A. baumannii*. Consequently, once *bla*$_{OXA}$-*mcr* co-carrying strains entered Enterobacterales, they disseminated efficiently within this order via these plasmids, ultimately forming dominant epidemic populations within Enterobacterales, while *A. baumannii* did not become a major transmission host.

Plasmid-mediated horizontal transfer and clone-mediated vertical transmission form a synergistic dissemination model. Previous studies have confirmed that the global spread of *bla*$_{OXA-48-like}$ genes relies on this synergistic mechanism: broad-host-range plasmids (e.g., IncL, IncX3) enable rapid cross-species dissemination of resistance genes, while high-risk clones (e.g., *Klebsiella pneumoniae* ST14, ST15, *Escherichia coli* ST38) provide stable genetic backgrounds that ensure sustained maintenance of resistance genes in dominant hosts. The Romania–UK transmission clone identified in this study (differing by only one SNP) further supports this synergistic model: the Col(MG828) plasmid provided the molecular tool for horizontal transfer, while the high genomic stability of this clone over a four-year period reflects its capacity for vertical transmission.

Beyond the synergy of transmission modes, the dissemination advantage of *bla*$_{OXA}$-*mcr* co-carrying strains is also reflected in their rich resistance gene payload. As revealed by the resistance gene co-linearity network in this study (Fig. 1D), *bla*$_{OXA}$-*mcr* co-carrying strains have generally accumulated a large and tightly interconnected "resistance gene reservoir," resulting in the breach of both "last-resort " defenses (carbapenems and colistin), often accompanied by resistance to various other antibiotic classes. This phenomenon is consistent with the "super-resistance island" observed in high-risk subclones of carbapenem-resistant *Klebsiella pneumoniae* ST11[37], suggesting that co-selection of multidrug resistance genes may be a common feature of such extremely drug-

resistant bacteria. Clones carrying such resistance gene reservoirs stably transmit core resistance genes through vertical inheritance, thereby enabling their continuous expansion across the human–animal–environment interface.

4. Co-evolution and structural simplification: Genetic basis for efficient - transmission

4. Co-evolution and structural simplification: Genetic basis for efficient *bla*$_{OXA}$-*mcr* transmission

Our results reveal marked differences in the transmission mechanisms of the two resistance gene classes across different bacterial species. In *Acinetobacter baumannii*, *bla*$_{OXA}$ genes are chromosome-borne intrinsic genes (belonging to the *bla*$_{OXA-51}$ family), while mcr genes are acquired via plasmid-mediated horizontal transfer (e.g., IncX1 plasmids), together conferring dual resistance. In contrast, among Enterobacterales, chromosome-borne mcr genes and plasmid-borne *bla*$_{OXA}$ genes exhibited similar temporal fluctuation patterns (Fig. 4B). This observation suggests a potential co-evolutionary mechanism: within Enterobacterales, strains carrying chromosome-borne *mcr* may serve as stable "recipient reservoirs" that, upon acquiring *bla*$_{OXA}$-carrying plasmids, form stably co-carrying dual-resistance phenotypes. A similar "vertical anchoring–horizontal acquisition" phenomenon has been observed previously: in a Czech Escherichia coli isolate, *bla*$_{OXA-244}$ was integrated into the chromosome while *bla*$_{NDM-5}$ was located on a plasmid, enabling stable co-carriage of two carbapenemase genes. This synergistic "vertical anchoring–horizontal acquisition" model may accelerate the population-level dissemination and maintenance of *bla*$_{OXA}$-*mcr* co-carrying strains and explains why this co-resistance phenotype continues to spread across different species and geographic regions.

Analysis of the flanking sequences of *bla*$_{OXA}$ genes revealed an important finding: none of the *bla*$_{OXA}$ genes in this study were located within known classical transposon structures (e.g., Tn1999, Tn2006) [22]; instead, they exhibited simplified genetic environments, such as the "*bla*$_{OXA-48}$–dmlA" unit. This structural feature suggests that these genes may rely primarily on plasmid backbones for overall dissemination rather than on independent transposition via flanking MGEs. Classical transposons typically carry multiple accessory genes, and their maintenance imposes certain fitness costs on the host[38]; in contrast, simplified genetic structures may reduce the metabolic burden on plasmids, thereby facilitating stable maintenance of these plasmids in host bacteria. By contrast, the flanking regions of *bla*$_{OXA-51}$ family genes—which are chromosome-borne intrinsic genes of *A. baumann*ii—display high diversity, reflecting their deep co-evolution with the host chromosome over long evolutionary periods. Furthermore, the observation of multi-copy formation of chromosome-borne *bla*$_{OXA-23}$ and related genes via replicative transposition in a clinical *A. baumannii* isolate[39] illustrates another evolutionary strategy by which bacterial genomes rapidly increase resistance levels through gene dosage effects. Together, these mechanisms confer strong genomic plasticity on *bla*$_{OXA}$-*mcr* co-carrying strains in responding to antibiotic pressure.

Compared with previous studies that have largely focused on single resistance genes (e.g., *mcr-1* or specific *bla*$_{OXA}$ variants) or specific regions[23, 40] the present study provides a global, systematic characterization of the complete landscape of strains co-carrying both classes of "last-resort " resistance genes. Our data not only confirm previous findings on the respective prevalence patterns of *bla*$_{OXA-48}$ and *mcr* genes but, more importantly, reveal through analysis of their co-carriage the potential pathways by which they achieve synergistic dissemination via specific plasmid combinations and clonal lineages.

Limitations

Although this study provides valuable insights, several limitations must be acknowledged. First, the analysis based on the NCBI database is subject to inherent reporting bias. The high proportion of strains from Germany (22.9%) is partially attributable to a focused hospital wastewater surveillance campaign in 2019[27], while sequencing data from many low- and middle-income countries remain scarce. Consequently, the distribution patterns observed in this study reflect global disparities in surveillance capacity rather than providing an absolute mirror of true prevalence. Second, this study is a retrospective genomic data analysis lacking corresponding clinical phenotypic data (e.g., minimum inhibitory concentrations, MICs) and patient outcome information, which limits its direct association with clinical treatment failure. Future studies should integrate genomic and phenomic data and strengthen active surveillance in under-monitored regions to establish a more balanced and comprehensive One Health surveillance system.

Conclusions

In summary, our study reveals that co-resistant strains carrying *bla*$_{OXA}$ and *mcr* genes have evolved into a global public health threat through “plasmid–clone”co-evolution and transmission across the human–animal–environment interface. The complexity of their transmission network and the “cryptic” nature of certain variants pose dual challenges to existing infection prevention and antimicrobial stewardship strategies. Addressing this threat requires a proactive, collaborative One Health approach: strengthening active surveillance of multidrug-resistant bacteria (particularly low-level resistance variants) globally; implementing stringent infection control measures in both healthcare and community settings to interrupt cross-species transmission chains; and ultimately advancing the development of novel antimicrobial agents and diagnostic technologies informed by resistance molecular epidemiology. Only through interdisciplinary and cross-sectoral collaboration can we fortify our defenses in the persistent battle against super-resistant bacteria.

Methods

1. Construction of the integrated dataset and basic characterization of strains

To systematically investigate the co-occurrence of *bla*$_{OXA}$ and *mcr* genes, we first retrieved bacterial genomes co-carrying both target gene classes from the NCBI database using the search query “AMR genotypes: *mcr* AND AMR genotypes: *bla*$_{OXA}$”. A total of 3,665 co-carrying strains were initially identified. To focus on *bla*$_{OXA}$ genotypes conferring carbapenem resistance, we compiled data from the BLDB database, identifying 722 *bla*$_{OXA}$ genes with confirmed carbapenem-hydrolyzing activity and downloading their reference sequences.

BLAST (v2.12.0) was used to align the sequencing data of the 3,665 genomes against the reference sequences of these resistance genes and the full set of *mcr* genes. The screening criteria were set as follows: >90% identity and > 90% coverage for *bla*$_{OXA}$ genes, and > 90% identity and > 80% coverage for *mcr* genes. Accordingly, 284 complete genome sequences meeting these criteria were identified, forming the core analysis dataset.

Strain metadata, including isolation year, country, source, and genus, were batch-extracted from GenBank (.gbff) format files using Python scripts. For 71 records with missing geographic information, the data were supplemented through literature cross-referencing[27]. Basic information for all strains is summarized in Supplementary Table 1. Based on these data, the spatiotemporal distribution, host sources, and other epidemiological characteristics of the strains were visualized and statistically analyzed using R software (packages ggplot2, rnaturalearth) and Python.

2. Phylogenetic analysis

To elucidate the evolutionary relationships and potential transmission clusters of *bla*$_{OXA}$-*mcr* co-carrying strains, we performed phylogenetic analysis on the 284 strains. A phylogenetic tree was constructed using GTDB-Tk software (default parameters) based on the 120 bacterial universal single-copy core gene marker set (Bac120). This marker set combines broad taxonomic coverage with moderate sequence conservation, enabling reliable inference of phylogenetic relationships among bacteria. The resulting phylogenetic tree was visualized and annotated using the iTOL online platform. Multiple layers of information were overlaid on the tree, including isolation year, country of origin, species classification, host source, and specific subtypes of *bla*$_{OXA}$ and *mcr* genes carried, thereby intuitively revealing the associations between strain evolution, geographic distribution, and resistance gene carriage patterns.

3. Identification of resistance gene localization and plasmid environment

To determine the genetic localization of *bla*$_{OXA}$ and *mcr* genes in the genomes, we first batch-extracted the genomic contig sequences containing the target genes, as confirmed by BLAST alignment, using Python scripts. Subsequently, PlasFlow—a tool based on deep neural networks (TensorFlow framework)—was employed to distinguish between chromosomal and plasmid origins of these sequences. For sequences predicted to be of plasmid origin, the PlasmidFinder database was further used to identify plasmid replicons (incompatibility groups, Inc types) and determine their plasmid types. Finally, the analysis results were integrated using a custom Python pipeline to clarify, for each strain, whether *bla*$_{OXA}$ and *mcr* genes were located on chromosomes or plasmids, and the results were visualized using R software (ggplot2) and other tools.

4. Analysis of the upstream and downstream structures of *bla*$_{OXA}$ genes

To further dissect the evolutionary relationships and characteristic distribution of co-carrying strains, we performed comprehensive, high-quality automated annotation on the 284 genomes using Bakta (v1.9.2), a professional bacterial genome annotation toolkit. Bakta (Bacterial Genome Annotation Toolkit) provides accurate gene prediction, functional annotation, and resistance gene identification. Based on its annotation results, we established a batch processing pipeline to perform in-depth analysis specifically targeting the upstream and downstream regions of *bla*$_{OXA}$ genes (typically covering a range of 5–10 kb), with a focus on characterizing the flanking mobile genetic elements (e.g., insertion sequences, transposons, integrons). This pipeline enabled fully automated analysis from raw genome sequences to the structural characterization of the genetic context surrounding resistance genes. Finally, the analysis results were organized using Python and visualized with R to display the common structures of mobile genetic elements harboring *bla*$_{OXA}$ genes.

Declarations

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval

Not applicable. This study did not involve human participants, their data, or biological material.

Consent to participate and consent to publish

Not applicable. This study did not involve human participants and does not contain any individual person's data or images.

Availability of data and materials

The genomic data analyzed in this study were obtained from the National Center for Biotechnology Information (NCBI) GenBank database. All raw sequencing data are publicly available and can be accessed via the NCBI Assembly database (https://www.ncbi.nlm.nih.gov/assembly/) using the accession numbers listed in Supplementary Table 1. The complete list of accession numbers and associated metadata (isolation year, country, source, and genus) is provided in the supplementary materials. The custom Python scripts used for data extraction and analysis are available from the corresponding author upon reasonable request.

Author`s contributions

BW: Writing – review & editing, Supervision, Resources, Conceptualization, Methodology, Funding acquisition. BA: Writing – review & editing,Writing – original draft, Validation, Visualization Data curation, Formal analysis Investigation. MC: Writing – review & editing, Formal analysis, Visualization, Investigation, Data curation. ZC: Writing – review & editing, Data curation, Resources, Formal analysis. XG: Data curation, Formal analysis, Writing – review & editing. LW: Writing – review & editing, Conceptualization. RZ: Writing – review & editing, Methodology, Visualization. JZ Methodology, Writing – review & editing. XZ: Writing – review &editing, Data curation . YO: Methodology, Writing – review & editing, Investigation. RW: Writing – review &editing, Data curation. TP:Writing – review &editing, Data curation. FW: Writing – review & editing, Data curation. QZ: Supervision, Investigation, Conceptualization, Writing– review & editing. HS: Supervision, Conceptualization, Writing – review & editing. HR: Supervision, Conceptualization, Writing – review & editing. XH: Supervision, Writing –review & editing, Conceptualization, Project administration, Funding acquisition.

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Figures

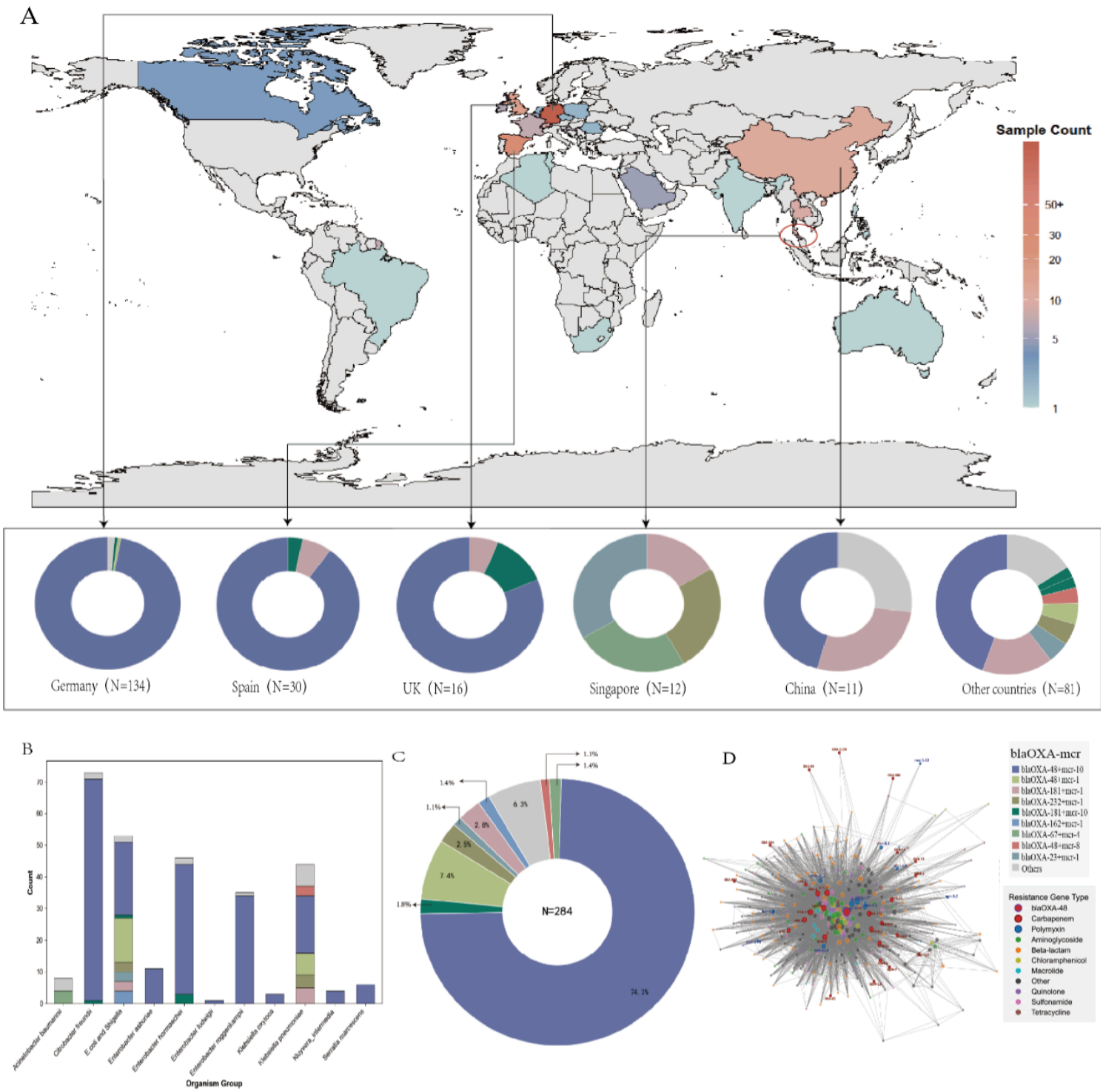


Figure 1

Global epidemiological distribution of *bla*$_{OXA}$–*mcr* co-carrying strains. A. Geographical distribution of 284 *bla*$_{OXA}$–*mcr* co-carrying strains worldwide from 2008 to 2024. The pie charts show the distribution of resistance genes in the top five countries with the highest number of isolates, as well as in other countries. **B.** Bacterial species distribution of resistance genes. **C.** Proportions of resistance gene combinations among the 284 *bla*$_{OXA}$–*mcr* co-carrying strains. **D.** Co-occurrence network of all antibiotic resistance genes in *bla*$_{OXA}$–*mcr* co-carrying strains. Nodes represent major antibiotic resistance genes, and edges connect nodes when two or more genes co-occur in the same isolate.

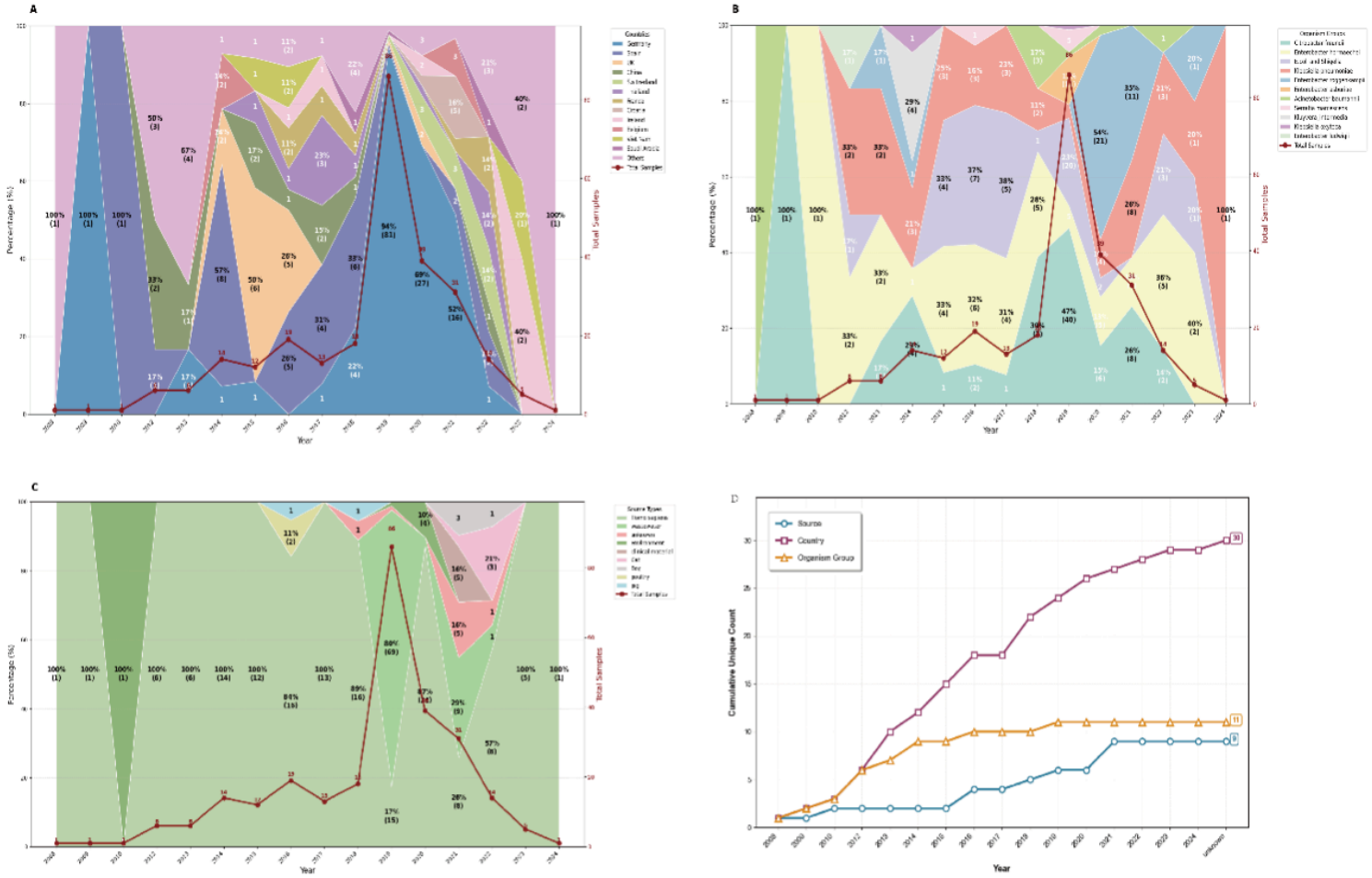


Figure 2

Analysis of the spatiotemporal transmission dynamics of *bla*$_{OXA}$–*mcr* co-carrying strains. A. Number of *bla*$_{OXA}$–*mcr* co-carrying strains collected in different countries over time. The x-axis indicates the year of collection. The left y-axis represents the percentage of strains collected from different countries each year. The line in the figure indicates the change in the total number of strains collected annually, with the right y-axis corresponding to the total count. **B.** Number of *bla*$_{OXA}$–*mcr* co-carrying strains collected from different bacterial species over time. The x-axis indicates the year of collection. The left y-axis represents the percentage of strains collected from different species each year. The line indicates the change in the total number of strains collected annually, with the right y-axis corresponding to the total count. **C.** Number of *bla*$_{OXA}$–*mcr* co-carrying strains collected from different sources over time. The x-axis indicates the year of collection. The left y-axis represents the percentage of strains collected from different host sources each year. The line indicates the change in the total number of strains collected annually, with the right y-axis corresponding to the total count. **D.** Cumulative changes in the number of sources, countries, and bacterial species over time.



Figure 3

Phylogenetic analysis of *bla*$_{OXA}$–*mcr* co-carrying strains. The phylogenetic tree was constructed based on whole-genome sequences of 284 bacterial strains co-carrying *bla*$_{OXA}$ and *mcr* genes. The outer circular annotations (from inner to outer layers) sequentially display the collection time, geographical origin, bacterial species, source, type of *bla*$_{OXA}$ gene carried, and type of *mcr* gene carried for each strain. The ● and ▲ symbols in the tree represent strains located within the same evolutionary clade but originating from different geographical sources, suggesting potential transregional events. These strains were randomly assigned into two groups, designated Group A and Group B, for SNP analysis, wherein ● denotes Group A and ▲ denotes Group B. The red label highlights the pair exhibiting the highest ANI value among the 12 groups.

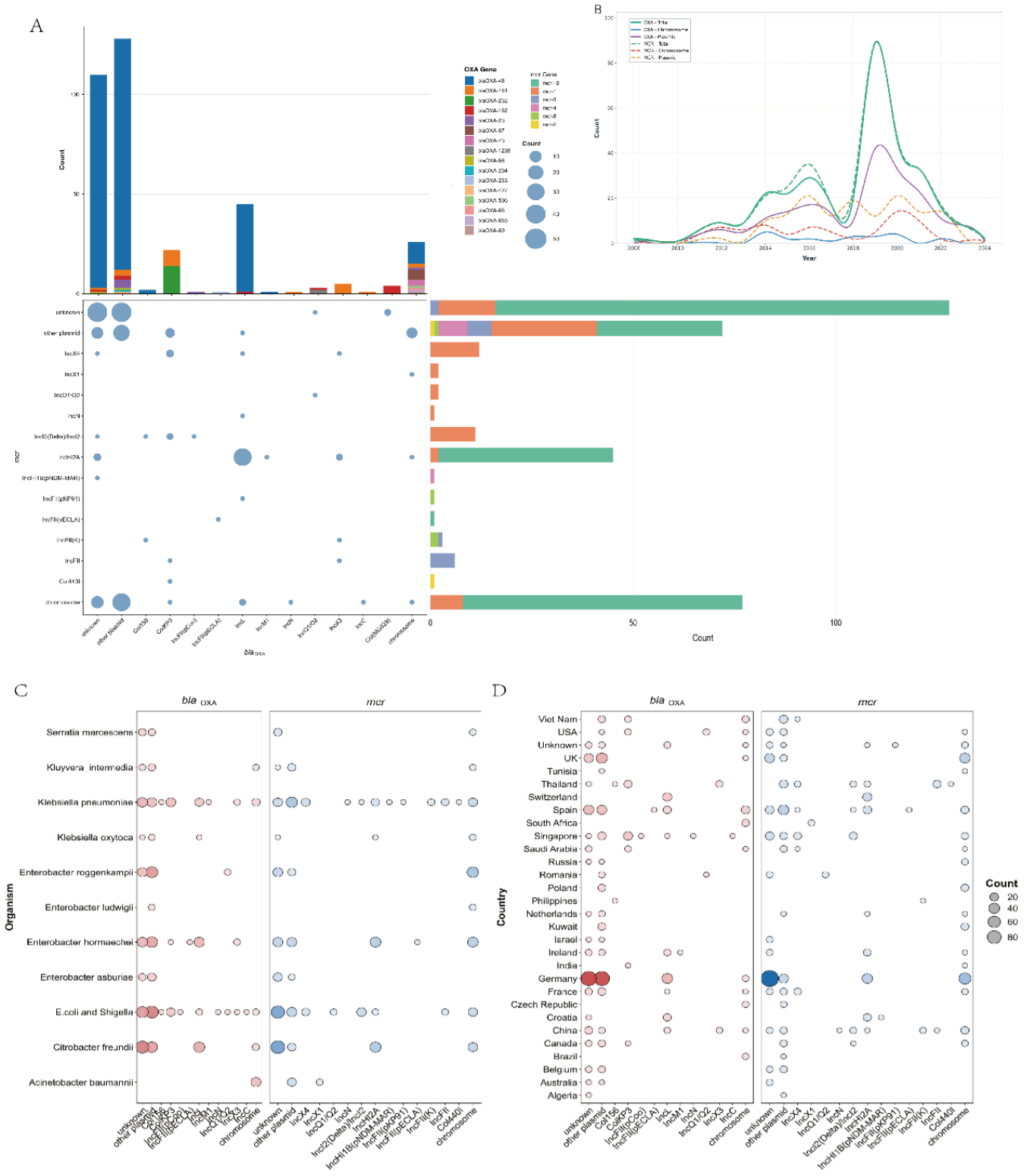


Figure 4

Characterization of plasmids carrying *bla*$_{OXA}$ and *mcr* genes in *bla*$_{OXA}$–*mcr* co-carrying strains. A. Distribution of combinations and counts of *bla*$_{OXA}$ and *mcr* genes on chromosomes and plasmid types across different co-carrying bacterial genera. **B.** Temporal changes of plasmids and chromosomes carrying *bla*$_{OXA}$ and *mcr* genes. **C.** Distribution of chromosomal and plasmid types carrying *bla*$_{OXA}$ and *mcr* genes across different bacterial species. **D.** Distribution of plasmids and chromosomes carrying *bla*$_{OXA}$ and *mcr* genes across different countries in co-resistant bacteria.

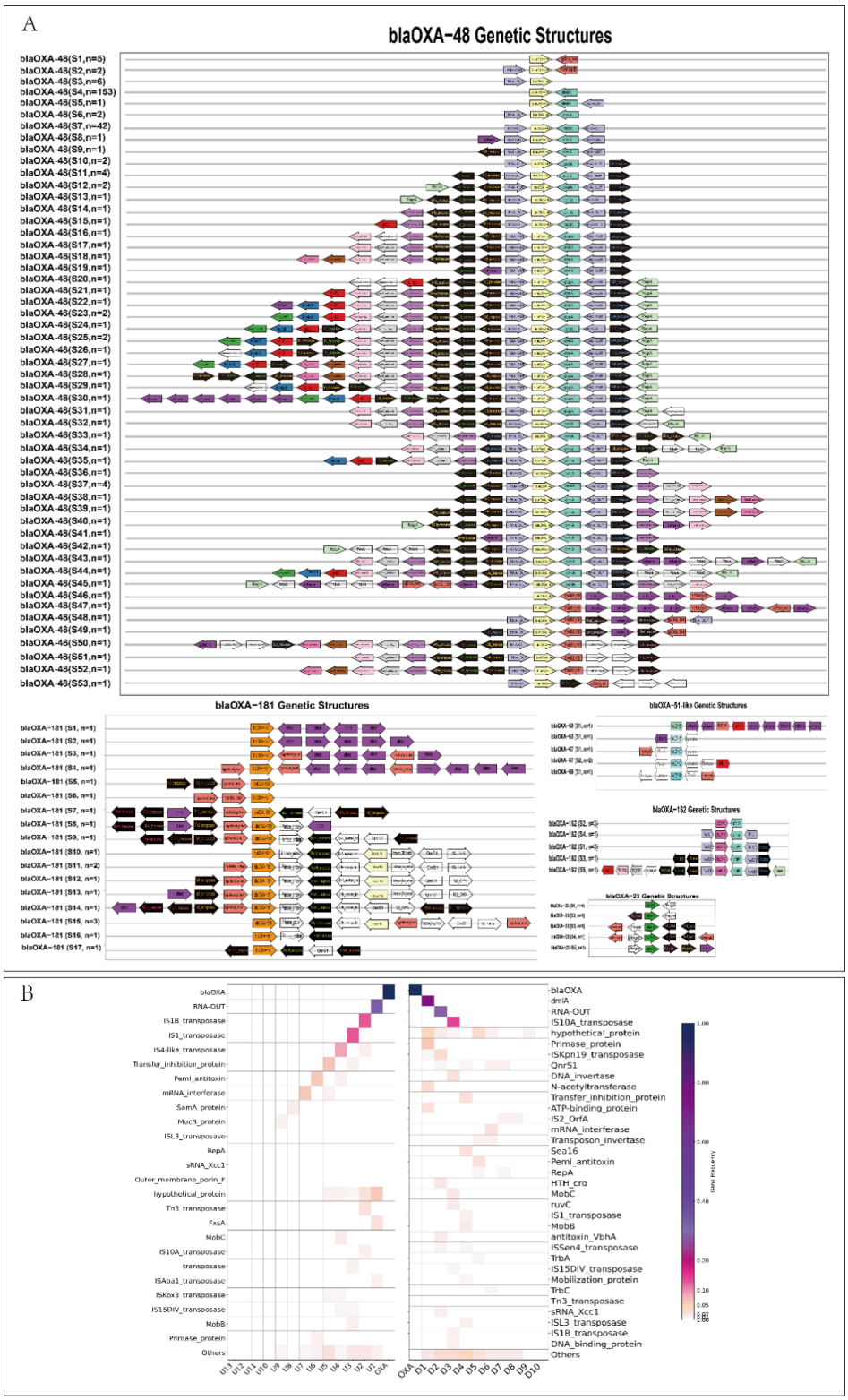


Figure 5

Detailed genetic context of *bla*$_{OXA}$ genes. A. Flanking sequence analysis of *bla*$_{OXA}$ genes. Arrows indicate direction and position only and are not drawn to scale. **B.** Heatmap of genetic element diversity upstream and downstream of *bla*$_{OXA}$ genes. Colors represent the percentage of strains in which the indicated structure appears at the corresponding position

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryfile2.csv
- supplementaryfile1.csv
- supplementarytable1.csv