

A One Health Approach Reveals Farms as Hotspots for Co-Occurrence of *Mcr-I* and *bla*_{NDM-1} Resistance Genes in Fuyang City, China

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Objective: The plasmid-mediated *mcr-I* and *bla*_{NDM-1} genes threaten the efficacy of last-line antibiotics. This study aimed to elucidate their prevalence and transmission risk within a “One Health” framework in Fuyang City, China, a region with intensive animal farming.

Methods: A total of 840 samples were collected and screened for *mcr-I* and *bla*_{NDM-1} using PCR, with confirmation by sequencing. DNA was extracted from all samples (directly from sample material for rat, pig, and poultry sources; from cultured isolates for clinical and food-derived strains). The samples comprised clinical strains (n=120), strains isolated from food samples (n=38), rat intestines (n=200), pig feces (n=194), and environmental swabs from live poultry markets (n=288).

Results: The *mcr-I* gene was prevalent (19.88%), particularly in pig feces (34.02%) and poultry environments (33.68%), while *bla*_{NDM-1} was less common (2.14%) but also highest in poultry environments (4.17%). Notably, we identified 25 samples (8.68% of poultry environments) that co-harbored both genes, constituting a concentrated hotspot for co-resistance. In stark contrast, clinical and food samples showed minimal to no detection of these genes. Statistical analysis confirmed significant differences in detection rates among sample types ($P < 0.05$).

Conclusion: Poultry farms are identified as crucial epicenters for the co-selection of *mcr-I* and *bla*_{NDM-1} in Fuyang. The high-density coexistence of these genes in a single environment poses a substantial public health risk, underscoring the urgent need for targeted interventions in the animal agriculture sector to prevent the transmission of pan-drug resistant bacteria from these reservoirs to human populations. This convergence within a farming environment suggests a high-risk interface for the emergence of pan-drug resistant pathogens, with potential for dissemination to humans via the food chain or environment, highlighting a region-specific One Health concern.

Keywords: one health, *mcr-I*, *bla*_{NDM-1}, co-resistance, poultry farm, antimicrobial resistance

Introduction

Antimicrobial resistance (AMR) poses a critical threat to global public health, compromising the effectiveness of conventional therapies.^{1,2} The emergence of plasmid-mediated mobile resistance genes, capable of horizontal transfer across diverse bacterial species, has exacerbated this crisis, presenting a higher risk than traditional resistance mechanisms.³ Among these, two genes are particularly alarming: *mcr-I* (*mobilized colistin resistance-I*), which confers resistance to the last-line antibiotic colistin,^{4,5} and *bla*_{NDM-1} (*New Delhi metallo-β-lactamase-I*), which hydrolyzes nearly all β-lactam antibiotics, including carbapenems.^{6,7} Since their discoveries, both genes have been reported worldwide across human, animal, food, and environmental samples,^{8–10} highlighting their pandemic potential.

The connection between antibiotic use in animal husbandry and the selection of resistance genes is well-established. The historical use of colistin as a growth promoter in farming is considered a significant driver for *mcr-1*.^{11,12} This intrinsically links human health to animal and environmental reservoirs, underscoring the necessity of a “One Health” perspective in combating AMR. While the individual prevalence of *mcr-1* and *bla*_{NDM-1} has been studied, their co-occurrence within integrated human-animal-environmental systems—especially in regional hotspots of agricultural production—remains poorly understood.¹³ Such co-occurrence is critical, as it creates a potential reservoir for the emergence of pan-drug resistant bacteria.

Fuyang City in Anhui Province, a key agricultural and livestock base in China, represents an ideal region to investigate this risk. The high density of farming activities and associated antibiotic use likely creates significant selective pressure for resistance gene emergence and spread. Therefore, this study aimed to determine the prevalence and distribution of *mcr-1* and *bla*_{NDM-1} across clinical, food, and environmental samples in Fuyang City, identifying specific hotspots for their co-occurrence to inform targeted interventions.

Materials and Methods

Study Design and Sampling

A “One Health” investigation was conducted in Fuyang City. An overview of the study design and sample processing workflow is illustrated in [Figure 1](#). A total of 840 samples were collected from five distinct sources between January 2023 and June 2024, as detailed in [Table 1](#). The sampling strategy employed convenience sampling at representative sites. Clinical and food-derived strains were isolated and identified as part of routine surveillance. Environmental and animal samples (rat intestines, pig feces, poultry environment) were collected fresh and processed for DNA extraction and bacterial culture. The research protocol was approved by the Ethics Committee of the Second People’s Hospital of Fuyang City (Approval No: 20231112041). Informed consent was obtained from human participants, and all personal identifiers were removed prior to analysis.

Bacterial Isolation

For clinical and food samples, strains were isolated on selective media as per standard laboratory protocols and preserved. For rat intestinal, pig fecal, and poultry environmental samples, approximately 0.1 g of sample (or 1 mL for liquid samples) was enriched in 5 mL of Luria-Bertani (LB) broth at 36°C with shaking (220 rpm) for 16–18 hours. A loopful of the enrichment culture was then streaked onto LB agar plates and incubated at 36°C for 18–24 hours to obtain isolated bacterial colonies for downstream analysis. This enrichment and plating step aimed to increase the likelihood of isolating bacteria carrying the target resistance genes, which may be present at low abundance in complex samples.

DNA Extraction

Bacterial genomic DNA was extracted from both the preserved clinical/food-derived isolates and the fresh cultures obtained from environmental/animal enrichments. For isolates, single colonies were inoculated into LB broth and cultured. For all samples, 1 mL of bacterial suspension was centrifuged at 12,000 rpm for 1 min. The pellet was processed using a commercial Bacterial Genomic DNA Extraction Kit (Tiangen Biotech, China) according to the manufacturer’s instructions. A 1 µL volume of the extracted DNA was used as the template for PCR amplification.

Detection of *Mcr-1* and *bla*_{NDM-1} Genes

Primers. Specific primers for *bla*_{NDM-1} (*bla*_{NDM-1}_17U-191: 5'-CAGCACACTTCCTATCTC-3' and *bla*_{NDM-1}_17L-465: 5'-CCGCAACCATCCCCCTCTT-3') and *mcr-1* (*mcr-1*_CLR5-F: 5'-CGGTCAGTCCGTTTGTTTC-3' and *mcr-1*_CLR5-R: 5'-CTTGGTCGGTCTGTAGGG-3') were used as previously described.¹⁴ The primer sequences and product sizes are summarized in [Table 2](#).

PCR Amplification. The 20 µL reaction mixture contained 1X Premix Taq™ (TaKaRa, China; containing 0.2 mM of each dNTP, 1.5 mM MgCl₂, and 1.25 U Taq polymerase), 0.25 µM of each primer, and 1 µL of DNA template. The same thermal

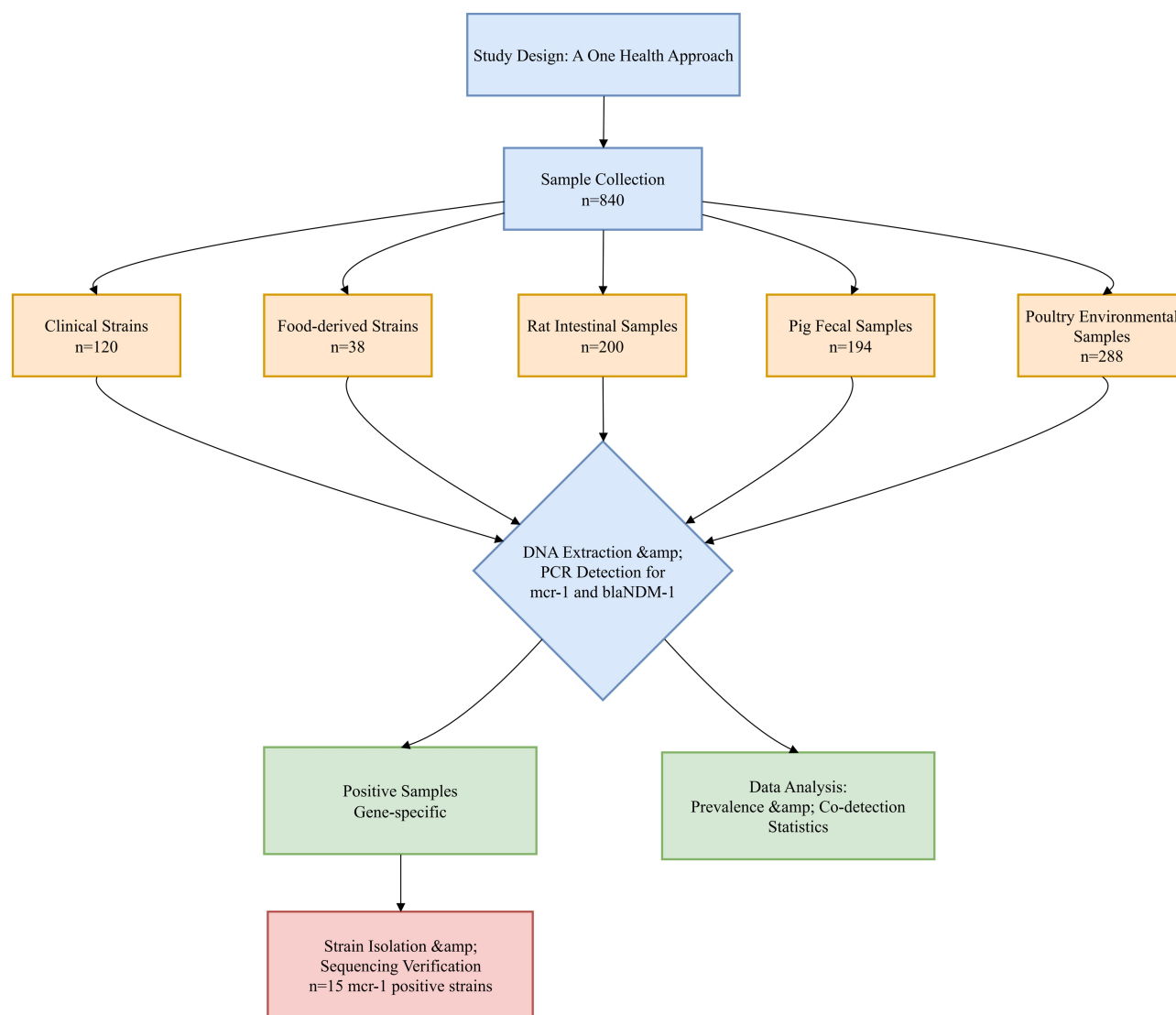


Figure 1 Flowchart of the study design.

cycling conditions were used for both genes: initial denaturation at 94°C for 5 min; followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min; with a final extension at 72°C for 5 min.

Electrophoresis and Sequencing. Amplification products (5 µL) were analyzed on a 1.5% agarose gel. A 292 bp product for *bla*_{NDM-1} and a 309 bp product for *mcr-1* indicated a positive result. All positive amplicons were sent for Sanger sequencing for confirmation.

Statistical Analysis

Statistical analyses were performed using SPSS 26.0. Differences in the detection rates of *mcr-1* and *bla*_{NDM-1} among the five sample categories were assessed using the Chi-square test. A P-value of less than 0.05 was considered statistically significant. Post-hoc pairwise comparisons with Bonferroni correction were conducted where applicable.

Results

Overall Detection of Resistance Genes

A total of 840 samples were tested in this study. 18 were positive for *bla*_{NDM-1}, with a positive rate of 2.14% (18/840). 167 were positive for *mcr-1*, with a positive rate of 19.88% (167/840).

Table 1 Summary of Sample Sources, Types, and Collection Details

Sample Category	Source/Description	Collection Site (County/District)	Collection Period	Number of Samples
Clinical Strains	Isolates from patients with foodborne/other infectious diseases	Fuyang People's Hospital	Jan – Dec 2023	120
Food-derived Strains	Isolates from retail food (pork, chicken, vegetables)	Local markets, Fuyang City	Jan – Dec 2023	38
Rat Intestinal Samples	Intestinal content from captured rats	Ying-shang County, Fu-nan County	Jan – Dec 2023	200
Pig Fecal Samples	Fresh feces from individual pigs	Four large-scale farms in Fu-nan County	Jan – Dec 2023	194
Poultry Environmental Samples	Environmental swabs (from cages, floors, feeding troughs) and chicken feces	Live poultry markets in four counties/districts of Fuyang City	Jan 2023 – Jun 2024	288
TOTAL				840

Note: Strains were identified as *Escherichia coli*, *Salmonella*, *Shigella*, *Acinetobacter baumannii*, *Cronobacter sakazakii*, and *Proteus mirabilis*.

Table 2 Primer Sequences, Product Lengths, and Annealing Temperatures for Gene Detection

Target Gene	Primer and Sequence (5'-3')	Product Length (bp)	Annealing Temp (°C)
<i>bla</i> _{NDM-1}	<i>bla</i> _{NDM-1} _17U-191:CAGCACACTTCCTATCTC <i>bla</i> _{NDM-1} _17L-465:CCGCAACCATCCCCTCTT	292	56
<i>mcr-I</i>	<i>mcr-I</i> _CLR5-F:CGGTCAGTCCGTTTGTTT <i>mcr-I</i> _CLR5-R:CTTGGTCGGTCTGTAGGG	309	56

Detection Rates and Co-Occurrence of Resistance Genes Across Sample Types

The detection rates of *mcr-I* and *bla*_{NDM-1} varied dramatically across the different sample categories, as detailed in Table 3. The overall prevalence of *mcr-I* (19.88%, 167/840) was significantly higher than that of *bla*_{NDM-1} (2.14%, 18/840). Strikingly, both genes were predominantly concentrated in farming environments. The highest positive rates for *mcr-I* were observed in pig fecal (34.02%, 66/194) and poultry environmental samples (33.68%, 97/288). Similarly, *bla*_{NDM-1} was also most frequently detected in these two categories, with positive rates of 3.09% (6/194) in pig feces and 4.17% (12/288) in poultry environments. In stark contrast, the detection rates in clinical, food-derived, and rat intestinal

Table 3 Detection Results of *Mcr-I* and *bla*_{NDM-1} Genes in Different Samples

Sample Category	Number of Samples	<i>bla</i> _{NDM-1} Positive No. (%)	<i>mcr-I</i> Positive No. (%)	Co-Detection Positive No. (%)
Clinical Strains	120	0 (0.00)	2 (1.67)	0 (0.00)
Food-derived Strains	38	0 (0.00)	0 (0.00)	0 (0.00)
Rat Intestinal Samples	200	0 (0.00)	2 (1.00)	0 (0.00)
Poultry Environmental Samples	288	12 (4.17)	97 (33.68)	25 (8.68)
Pig Fecal Samples	194	6 (3.09)	66 (34.02)	0 (0.00)
Total	840	18 (2.14)	167 (19.88)	25 (2.98)

Note: Data were analyzed by Chi-square test. The differences in detection rates among the five sample categories were statistically significant ($P < 0.001$ for both *bla*_{NDM-1} and *mcr-I*).

samples were very low or zero. Statistical analysis confirmed that the differences in detection rates among sample types were highly significant for both genes ($P < 0.05$, Chi-square test).

Most critically, further analysis revealed the co-occurrence of *mcr-1* and *bla*_{NDM-1} genes in 25 samples. These double-positive samples were exclusively identified in poultry environments, representing 8.68% (25/288) of all poultry environmental samples and underscoring this niche as a specific hotspot for the convergence of critically important resistance mechanisms.

Isolation and Identification of Positive Strains

To obtain bacterial isolates harboring the resistance genes, we attempted isolation from a subset of PCR-positive samples. Bacterial isolation was attempted from a subset of 50 PCR-positive samples, prioritizing samples with strong PCR signals and encompassing all sample categories where positives were found (ie, pig feces, poultry environment, and rat intestine). From this subset, a total of 15 strains carrying the *mcr-1* gene were successfully isolated and identified through 16S rRNA gene sequencing and subsequent *mcr-1* gene confirmation. The isolates comprised 12 non-diarrheagenic *Escherichia coli*, 1 diarrheagenic *Escherichia coli*, 1 *Acinetobacter baumannii*, and 1 *Leclercia adecarboxylata*.

These isolates originated from the following sources:

1. Pig feces: 10 isolates (all *E. coli*)
2. Poultry environmental samples: 4 isolates (3 *E. coli*, 1 *A. baumannii*)
3. Rat intestinal sample: 1 isolate (*L. adecarboxylata*)

No *bla*_{NDM-1}-positive isolates were successfully recovered from the culture attempts.

Note on gene sequence: The *mcr-1* gene sequences amplified from these isolates were 100% identical to known reference sequences in GenBank (eg, Accession MK965883, MK965884) for *mcr-1*, and the *bla*_{NDM-1} gene sequences detected by PCR were 100% identical to the reference NDM-1 sequence (Accession WP_004201164.1). Therefore, to avoid redundancy of identical sequences in public databases, the novel isolates have been preserved and are available upon request, while the gene sequences have been authenticated by alignment with these established references.^{15,16}

Discussion

The global spread of plasmid-mediated resistance genes, such as *mcr-1* and *bla*_{NDM-1},^{17–19} represents a critical frontier in the fight against antimicrobial resistance (AMR) due to their horizontal transferability among diverse bacterial hosts. This study presents a systematic, One Health-based investigation into the prevalence of these two critical genes within the distinct ecological context of Fuyang City, a major agricultural region in China. Our findings reveal a clear and concerning epidemiological pattern: both genes are highly entrenched in animal farming environments, with a significant risk of co-selection, while currently demonstrating limited dissemination into human clinical settings.

The most striking feature of our results is the pronounced disparity in the ecological distribution of *mcr-1* and *bla*_{NDM-1}. The exceptionally high detection rates of *mcr-1* in pig feces (34.02%) and poultry environments (33.68%) are consistent with the historical use of colistin as a growth promoter in livestock farming, creating a potent selective pressure.^{20–22} The concurrent, though lower, prevalence of *bla*_{NDM-1} in the same environments (3.09% in pig feces; 4.17% in poultry environments) suggests that these farming systems are subject to broad antibiotic pressures, facilitating the maintenance of diverse resistance determinants. In stark contrast, the very low detection rate in clinical (<1.67%) and food-derived (0%) samples indicates that, for now, the human health burden directly attributable to these genes in this region remains limited. This pattern strongly suggests that animal farms act as the primary reservoirs, with the clinical cases likely representing sporadic introduction events rather than sustained human-to-human transmission.²³ The low prevalence in rat intestines further suggests that wild rodents are not primary reservoirs but may act as passive sentinels of environmental contamination.

The most critical finding of this study is the exclusive co-occurrence of *mcr-1* and *bla*_{NDM-1} genes in 25 poultry environmental samples (8.68% of this category). The confinement of these two last-line antibiotic resistance genes to a single ecological niche suggests a “genetic hotspot” where bacterial populations are continuously exposed to co-

selective pressures. It is important to interpret this finding within the methodological context: while PCR effectively identifies the co-occurrence of resistance genes in a sample, it does not confirm their co-localization on the same mobile genetic element within a single bacterial cell. Nonetheless, such a high-density convergence creates a plausible environment that may facilitate the acquisition of both genes by a single bacterial clone, which could give rise to pan-drug resistant pathogens—a scenario with documented clinical implications.²⁴

Our environmental finding resonates with and extends reports from clinical settings. For instance, Tian et al (2020)¹³ isolated an *E. coli* ST27 strain co-harboring *bla*_{NDM-1}, *mcr-1*, and *fosA3* from a patient in China, demonstrating the clinical reality and severity of such co-resistance. While that report focused on a human isolate, our study pinpoints a potential farm-based environmental reservoir from which such pathogens may emerge. The significant therapeutic challenge posed by bacteria with combined *mcr-1* and *bla*_{NDM-1} resistance is further underscored by ongoing research into strategies to overcome this specific resistance profile.²⁵ The pronounced disparity in gene prevalence—with both genes highly entrenched in animal farms but rare in clinical samples—corroborates the well-established link between historical colistin use in livestock as a growth promoter and the selection of *mcr-1*,^{26,27} solidifying the role of animal farms as primary resistance reservoirs.

The successful isolation and identification of 15 *mcr-1*-positive strains (including *E. coli*, *A. baumannii*, and *L. adecarboxylata*) from various samples confirm the mobility of this gene across different bacterial species. This genetic mobility, coupled with the high density of resistance genes in farming environments, underscores the potential for these reservoirs to serve as sources for community and clinical transmission through routes such as environmental contamination, food chain exposure, or direct contact.

Limitations

A limitation of this study is that its PCR-based screening approach, while ideal for large-scale surveillance, did not include phenotypic susceptibility testing or molecular experiments to confirm the transferability of the resistance genes. However, the clear identification of high-risk hotspots, particularly the samples co-harboring both genes, provides a strong rationale and prioritizes specific targets for such future mechanistic studies. The preserved isolates represent a valuable resource for subsequent whole-genome sequencing, plasmid analysis, and conjugation experiments to elucidate the precise transmission mechanisms.

Conclusion

In conclusion, this One Health investigation provides strong evidence identifying animal farming environments, particularly poultry farms, as key reservoirs and convergence points for *mcr-1* and *bla*_{NDM-1} genes in Fuyang City. The significant co-occurrence of these critical resistance determinants in a single niche represents a potential risk for the emergence and dissemination of multidrug-resistant pathogens. These findings underscore the urgent need to implement targeted interventions, including strict antibiotic stewardship in livestock production and integrated surveillance programs bridging human, animal, and environmental health sectors. Future research should prioritize genomic analysis of isolates from these identified hotspots to elucidate the precise mechanisms of gene transfer and confirm co-localization, which will be crucial for designing effective containment strategies.

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Disclosure

The authors report no conflicts of interest in this work.

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