



Original Article

## Molecular characterization of emergence of mobile colistin resistance 1 gene-mediated colistin resistance in clinical isolates of *Escherichia coli*: A concern from North India

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### ABSTRACT

**Objectives:** Colistin is a reserved, last-resort antibiotic used to treat infections caused by multidrug-resistant Gram-negative bacilli. The mobile colistin resistance (*mcr-1*) gene has been identified as a key mechanism for colistin resistance. This study aimed to investigate the association between the *mcr-1* gene and colistin resistance in *Escherichia coli*.

**Methods:** A total of 1,149 clinical isolates of *E. coli* were collected over 1 year from various clinical specimens at a tertiary care center in North India. Initial colistin susceptibility testing was performed using the automated VITEK 2 system, followed by confirmation of resistance by the gold standard broth microdilution (BMD) method. Isolates confirmed as resistant by BMD were further screened for the *mcr-1* gene by real-time polymerase chain reaction. The data were analyzed using SYSTAT software version 13.2.

**Results:** Out of the total *E. coli* isolates, 77.5% was obtained from hospitalized patients. Colistin resistance was initially detected in 11 (0.95%) isolates, of which 9 (0.78%) were confirmed by BMD. The *mcr-1* gene was detected in three (33.3%) of these nine phenotypically confirmed resistant isolates. None of the non-resistant isolates tested harbored the *mcr-1* gene.

**Conclusion:** The emergence of colistin-resistant *E. coli* is a clinically important concern because it limits treatment options. Detection of *mcr-1*-positive isolates supports the need for continued antimicrobial surveillance, stewardship, and infection-control vigilance.

**Keywords:** Broth microdilution, Colistin, *Escherichia coli*, *mcr-1* gene, Tertiary care hospital

### INTRODUCTION

Gram-negative bacilli (GNB) are major causes of human infection. Among GNBs, *Escherichia coli*, a member of the genus *Escherichia*, is a major pathogen known to cause a wide range of infections, including urinary tract infection, skin and soft tissue infection, septicemia, endotoxic shock, pneumonia, and meningitis in neonates.<sup>[1]</sup>

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A well-recognized issue that frequently renders empirical therapy ineffective is multidrug resistance (MDR). Infections caused by MDR GNBs can be effectively treated with polymyxins, such as colistin. However, their clinical application is restricted by their inadequate urinary clearance and potential adverse effects, such as neurotoxicity and nephrotoxicity.<sup>[2,3]</sup> Colistin is being reconsidered as a last-resort treatment option due to the growing prevalence of MDR Gram-negative pathogens, especially those that show carbapenem resistance.<sup>[4]</sup> The use of colistin has grown as an efficient treatment option due to the increased prevalence of MDR Gram-negative infections, particularly in India.<sup>[5-8]</sup>

Colistin resistance has arisen as a result of the frequent use of colistin to treat these MDR pathogens. In accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing and the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antimicrobial Susceptibility Testing (AST), isolates should be reported as colistin-resistant only after confirmation by broth microdilution (BMD) using colistin sulfate, which is the reference method for colistin susceptibility testing.<sup>[9]</sup> The lack of the reference method in many laboratories, however, may result in inaccurate data and an underestimation of the true prevalence of colistin resistance among MDR GNBs.

Several mechanisms have been proposed for *E. coli*; the emergence of colistin resistance has been attributed to a variety of mechanisms, including genetic mechanisms. The mobile colistin resistance (*mcr*) gene is one of the most important ones. The most frequently linked genes to colistin resistance among the *mcr* variants (*mcr-1* to *mcr-10*) is *mcr-1*.<sup>[3]</sup> There is currently a lack of information on the genetic characterization of colistin resistance in this geographic area. Hence, the aim of this study was to find how the *mcr-1* gene is linked to *E. coli* strains that are resistant to colistin in North Indian tertiary care hospital.

## MATERIALS & METHODS

### Study design and setting

This cross-sectional hospital-based observational study was conducted in the Department of Microbiology at a tertiary care hospital over a period of 1 year, from October 2023 to September 2024. Clinical samples from patients with suspected infection were collected according to sample-collection guidelines. Samples meeting the inclusion criteria were included in the study. Data were entered into a Microsoft Excel spreadsheet after application of the inclusion and exclusion criteria.

### Inclusion criteria

The clinical samples from patients of all age groups and genders showing the significant growth of *E. coli* on culture

from both inpatient department (IPD) and outpatient department (OPD) were included in the study. In case of multiple isolations from the same patient, the *E. coli* isolated first was included in the study.

### Exclusion criteria

The bacterial isolates other than *E. coli* were excluded from the study. In case of multiple isolates from the same patient, subsequent *E. coli* isolates were also excluded from the study. The patients with recent antibiotic exposure were excluded from the study.

The final dataset was compiled in a Microsoft Excel spreadsheet, following the inclusion and exclusion criteria.

### Sample processing for isolation and identification of aerobic bacteria

The clinical specimens were processed for aerobic culture on appropriate culture media following standard bacteriological techniques.<sup>[10]</sup> The growth on solid culture media was subjected to preliminary identification based on colony characteristics and Gram staining. The final species-level identification and AST were performed by the VITEK 2 compact automated system (bioMérieux, France) using the GN test card and AST-N405 cards, respectively.

### BMD method for detection of colistin resistance

Colistin resistance was confirmed by the reference BMD method performed in accordance with CLSI M07-A10 guidelines, 10<sup>th</sup> edition, 2015, using cation-adjusted Mueller-Hinton broth (CaMHB), and interpretation of minimum inhibitory concentration (MIC) was done as per CLSI M100 guidelines, 33<sup>rd</sup> edition, 2023.<sup>[11,12]</sup> Briefly, colistin sulfate (HiMedia India Pvt. Ltd., Mumbai) was used to prepare colistin concentrations by serial two-fold dilutions in CaMHB, ranging from 0.25 to 64 µg/mL.<sup>[13]</sup> A bacterial suspension was added to each well to achieve a final inoculum of 10<sup>5</sup> colony-forming units/mL. A drug-free control (growth control) and a non-inoculated control (sterility control) were included in each assay for quality control. *E. coli* ATCC 25922 was tested each time as a negative control (NC) with expected MIC breakpoints of 0.5–2 µg/mL. The MICs were determined after overnight incubation at 37°C. Interpretation of MIC was based on CLSI breakpoints for enterobacterales. Isolates with MIC value of ≥4 µg/mL were classified as resistant, while those with MIC ≤2 µg/mL were considered non-resistant as mentioned in CLSI M-100 guidelines 2023 (33<sup>rd</sup> Edition).<sup>[12]</sup>

### Genotypic characterization of colistin resistance

The association of colistin resistance with plasmid-mediated transferable *mcr* genes has been reported in the literature.

Out of the *mcr-1* to *mcr-10* variants of *mcr* genes studied so far, *mcr-1* is the most commonly associated variant with colistin resistance. The other *mcr* variants, *mcr-2* to *mcr-10*, are also associated with colistin resistance to a lesser extent only; our study was limited to finding the association of only the *mcr-1* gene with colistin resistance.

### Detection of the *mcr-1* gene by real-time polymerase chain reaction (PCR)

All phenotypically confirmed colistin-resistant *E. coli* isolates, along with 1% of known colistin non-resistant isolates, were subjected to real-time PCR for detection of the *mcr-1* gene for quality control.

Genomic deoxyribonucleic acid (DNA) was extracted using the GeNei Pure Bacterial DNA Purification Kit (GeNei Laboratories Pvt. Ltd., Bengaluru, India) according to the manufacturer's instructions. Extracted DNA was stored at  $-20^{\circ}\text{C}$  until use. Amplification was performed using the GeNei™ PCR kit according to the manufacturer's guidelines.

Specific forward and reverse primers used for *mcr-1* gene detection are shown in Table 1.<sup>[14]</sup>

The final volume for the PCR reaction was 25  $\mu\text{L}$ , consisting of 3  $\mu\text{L}$  of nuclease-free water, 12  $\mu\text{L}$  of EvaGen™ Green quantitative PCR (qPCR) Master Mix, 2  $\mu\text{L}$  of working primers at a final concentration of 5 pmol, and 3  $\mu\text{L}$  of template DNA. PCR was carried out following EvaGen™ Green qPCR guidelines (GeNei Laboratories Pvt. Ltd., Bengaluru, India). PCR cycling conditions included an initial denaturation at  $95^{\circ}\text{C}$  for 3 min, followed by 35 cycles of  $95^{\circ}\text{C}$  for 15 s, primer annealing at  $58^{\circ}\text{C}$  for 30 s, and elongation at  $72^{\circ}\text{C}$  for 1 min, with a final extension at  $72^{\circ}\text{C}$  for 15 min. Amplification was carried out using the Rotor-Gene Q system (QIAGEN, Germantown, MD, USA). DNA from colistin-susceptible *E. coli* ATCC 25922 served as the NC for the *mcr-1* gene. A standard strain harboring the *mcr-1* gene was used as the positive control.

### Statistical analysis

Data generated in this study were analyzed using SYSTAT version 13.2 and the Statistical Package for the Social Sciences software 27.0 (IBM Corp., USA). Descriptive statistics,

Primer	Primer sequences	Product bp
Forward primer	5'-CGTTCAGCAGTCATT ATGCCAGTTTCTTTCGCGTGC-3'	956 bp
Reverse primer	5'-CTTACGCATATCAGGC TTGGTTGCTTGTACCGC-3'	956 bp

A: Adenine, T: Thymine, C: Cytosine, G: Guanine

including frequency and percentage, were used to summarize the sample characteristics. Fisher's exact test was used to calculate the *p*-value and the odds ratio. Fisher's exact test was used to assess the association between hospital location and colistin resistance. Odds ratios with 95% confidence intervals were calculated to estimate the effect size. A  $p < 0.05$  was considered statistically significant.

## RESULTS

### Phenotypic characterization of colistin-resistant *E. coli* isolates

A total of 1,149 clinical isolates of *E. coli* were included in the study. On antibiotic susceptibility testing, resistance to colistin was detected in 11 (0.95%) isolates by an automated method, and resistance was confirmed by the gold standard BMD method in 9 (0.78%) isolates as shown in Table 2. The microtiter plate showing the results of BMD is shown in Figure 1.

### Sample-wise and gender wise distribution of colistin resistance

As illustrated in Table 3, urine samples accounted for the majority of colistin-resistant *E. coli* isolates (54.5%), followed by pus samples (36.3%) and endotracheal aspirate (9.09%). Most of these isolates (72.7%) were recovered from patients admitted to the IPD and intensive care units (ICUs). With 63.3% of cases, males outnumbered females in terms of gender distribution, and the ratio of male-to-female was 1.75:1.

### Genotypic characterization of colistin resistance

The presence of the *mcr-1* gene was ascertained by genotypic characterization of 11 clinical isolates of *E. coli*, nine of

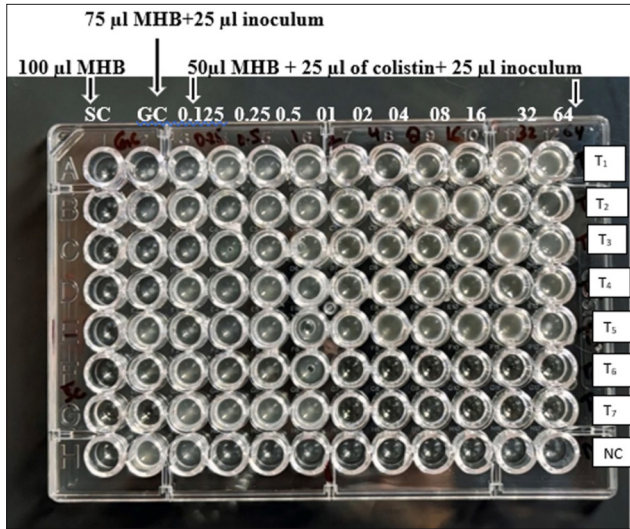
**Table 2:** Distribution of colistin-resistant isolates of *Escherichia coli* by VITEK 2 and BMD ( $n=1149$ ).

<i>Escherichia coli</i> isolates	Colistin resistance by automated VITEK 2	Colistin resistance by BMD
Frequency	11	9
Percentage	0.95	0.78

BMD: Broth microdilution

**Table 3:** The distribution of colistin resistance in *Escherichia coli* isolates by sample type ( $n=11$ ).

Sample	Colistin-resistant isolates (Frequency)	Percentage
Urine	6	54.5
Pus	4	36.4
Endotracheal aspirate	1	9.09



**Figure 1:** Broth microdilution plate used for the determination of colistin minimum inhibitory concentration in *Escherichia coli* isolates. SC: Sterility control, GC: Growth control, T1-T7: Test isolates, NC: Negative control, MHB: Mueller Hinton Broth.

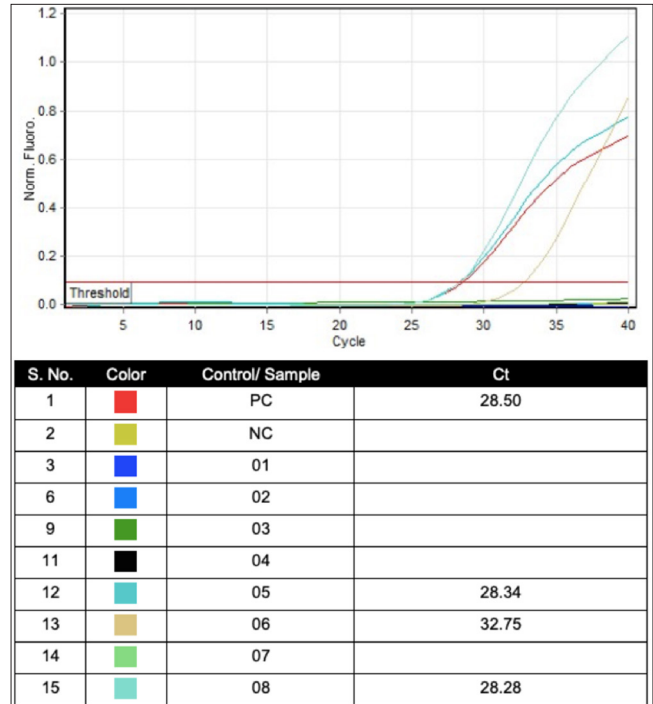
which were confirmed by BMD. Only three (33.3%) *E. coli* isolates contained the *mcr-1* gene, as shown in Table 4. The amplification curves showing positive and negative results for the *mcr-1* gene are shown in Figure 2. However, the *mcr-1* gene was not detected in eight phenotypically colistin-resistant isolates or in any of the colistin non-resistant *E. coli* isolates tested.

### Hospital location-wise distribution of colistin-resistant isolates

The majority of the colistin-resistant *E. coli* isolates were recovered from the ICU and IPD as compared to OPD. The comparative distribution of all three methods based on hospital location is shown in Table 5. The genotypically confirmed *E. coli* isolates harboring *mcr-1* were exclusively recovered from the ICU patients. The total number of *E. coli* isolates and distribution of colistin-resistant *E. coli* isolates, along with the statistical parameters, are shown in Table 6.

## DISCUSSION

The emergence and rising trends of antimicrobial resistance constitute a global challenge and pose a serious threat to healthcare systems worldwide, primarily due to the injudicious use and misuse of antimicrobials.<sup>[15]</sup> Colistin is often regarded as a last-resort antibiotic. It is now used to treat infections caused by bacteria that are resistant to multiple antibiotics. However, the emergence of colistin resistance in clinical isolates has become a significant therapeutic concern.<sup>[15,16]</sup>



**Figure 2:** Real-time amplification curves for detection of the *mcr-1* gene in *Escherichia coli* isolates. PC: Positive control; NC: Negative control; Ct: Cycle threshold.

**Table 4:** Distribution of the *mcr-1* gene in BMD confirmed colistin-resistant isolates ( $n=9$ ).

Colistin-resistant isolates by BMD	Colistin-resistant isolates of <i>Escherichia coli</i> harboring <i>mcr-1</i> gene (frequency)	Percentage
9	3	33.3

BMD: Broth microdilution

**Table 5:** Hospital location-wise distribution of colistin-resistant *Escherichia coli* isolates using various methods.

Hospital location	Automated method ( $n=11$ )	Broth microdilution ( $n=9$ )	Genotypic method ( <i>mcr-1</i> ) ( $n=3$ )
ICU	5	5	3
Other wards	3	3	0
OPD	3	1	0

ICU: Intensive care unit, OPD: Outpatient department

In the present study, we observed 0.78% colistin-resistant strains of *E. coli* using the gold standard BMD method, which is considerably lower than the 4.2% reported in a study by Arjun *et al.* from South India.<sup>[17]</sup> Although the prevalence of confirmed colistin resistance in this study was low, the detection of *mcr-1*-positive isolates indicates

**Table 6:** Association between ICU location and colistin resistance (*n*=1149).

Hospital location	Colistin non-resistant ( <i>n</i> =1140)	Colistin resistant ( <i>n</i> =9)	<i>p</i> -value	Odds ratio with 95% CI
ICU	241	5	0.012	4.66 (1.24–17.50)
Non-ICU	899	4		

ICU: Intensive care unit, CI: Confidence interval

the presence of plasmid-mediated resistance and supports continued surveillance.<sup>[18]</sup> Therefore, continuous surveillance is important to prevent further dissemination of resistant strains. Reliable phenotypic detection of colistin resistance before clinical use is essential using BMD, as BMD is a gold-standard, cost-effective, and reliable method for the routine detection of colistin resistance.<sup>[9]</sup>

In addition, the presence of the *mcr-1* gene in 33.3% of phenotypically confirmed colistin-resistant isolates of *E. coli* in our study is a significant concern. Singh *et al.* from Himachal Pradesh reported a prevalence of the *mcr-1* gene in only 5.9% of *E. coli* isolates that exhibited colistin resistance.<sup>[19]</sup>

The absence of the *mcr-1* gene in 66.7% of phenotypically confirmed colistin-resistant *E. coli* isolates in our study suggests that *mcr-1* is not the sole mechanism responsible for colistin resistance. Other genetic determinants or mechanisms may be contributing to resistance in these isolations. In addition, the presence of other *mcr* variants (*mcr-2* to *mcr-10*) cannot be excluded. One of the major limitations of this study was that we focused solely on the detection of the *mcr-1* gene, despite the existence of multiple *mcr* genes associated with colistin resistance.<sup>[15]</sup> These findings highlight the scope for future research to explore other resistance genes, gene-to-gene interactions, and a whole-genome sequencing approach to identify other genetic variants responsible for colistin resistance.

As the *mcr-1* gene is plasmid-mediated, its potential for horizontal gene transfer raises significant concern regarding rapid dissemination, particularly within hospital settings.<sup>[2]</sup> Identification of isolates harboring plasmid-mediated *mcr-1* genes is therefore crucial for understanding regional resistance trends. Horizontal gene transfer is not limited to hospital settings; environmental bacteria have also been shown to harbor the *mcr-1* gene, posing a risk of transmission to other human pathogens and serving as a reservoir capable of triggering outbreaks.<sup>[6,18]</sup> Early identification of such isolates can help in preventing nosocomial outbreaks through targeted infection prevention and control measures. In this context, real-time PCR can provide rapid and accurate detection of the *mcr-1* gene in comparison to conventional phenotypic methods.<sup>[20]</sup>

Limited Indian data on *mcr-1* gene-based detection of colistin resistance in clinical isolates of *E. coli* restrict direct comparisons. However, international studies report variable prevalence of the *mcr-1* gene, often higher than that observed in our study. Cannatelli *et al.* reported the presence of the *mcr-1* gene in 88.8% of phenotypically colistin-resistant *E. coli* isolates in a study from Italy, while Liu *et al.* reported 63% prevalence of the *mcr-1* gene.<sup>[6,21]</sup> In contrast, a Latin American study by Rapoport *et al.* documented a much lower prevalence of 10.3%.<sup>[8]</sup> These variations may be attributed to alternative resistance mechanisms, including phenotypic adaptations and genetic determinants other than *mcr-1*. Nevertheless, *mcr-1* remains the most common and frequently reported genetic element associated with colistin resistance in *E. coli*, as reported by Shi *et al.* They reported *mcr-1* in 86.1%, followed by *mcr-9* in 5.7% of colistin-resistant strains of *E. coli*.<sup>[22]</sup>

The colistin-resistant *E. coli* strains were most commonly isolated from the patients admitted to ICUs, followed by other IPD settings, as compared to OPD. These trends indicate that hospital-acquired strains, especially the strains from ICUs, are more resistant than community-acquired strains. On comparing ICU and non-ICU *E. coli* isolates statistically, colistin resistance was significantly associated with ICU isolates as indicated by a statistically significant  $p = 0.012$  ( $<0.05$ ) and an odds ratio of 4.66, indicating that isolates from ICU patients had higher odds of colistin resistance than those from non-ICU patients.

All *mcr-1*-harboring colistin-resistant *E. coli* isolates in our study were recovered from patients admitted to ICUs. These results are consistent with the hospital antibiogram, which highlights the MDR nature of strains frequently associated with hospital-acquired infections during ICU stay. Collectively, these findings underscore the need for targeted surveillance and stringent infection prevention and control measures in high-risk healthcare settings.

Another critical concern is the under-reporting or over-reporting of colistin resistance due to the non-availability of recommended detection methods in many clinical laboratories.<sup>[6]</sup> Further studies using gold-standard phenotypic methods combined with molecular characterization of resistance mechanisms are essential to accurately determine the actual burden of colistin resistance.

In recent years, a gradual increase in colistin resistance has been observed, further exacerbated by the plasmid-mediated nature of the *mcr* genes, facilitating rapid horizontal gene transfer across diverse bacterial species.<sup>[4]</sup>

The emergence of colistin-resistant *E. coli* in North India is a trend that has substantial therapeutic and public health implications, as our study has demonstrated. Colistin resistance is conferred by genetic mechanisms, of which the *mcr-1* gene is one of the most prevalent contributors. Overall,

the results highlight how urgently molecular surveillance and an all-encompassing approach are needed to combat the rising threat of antibiotic resistance in India.

### Limitations of the study

This study had certain limitations. First, the analysis was restricted to the detection of the *mcr-1* gene only; other *mcr* gene variants (*mcr-2* to *mcr-10*), as well as the plasmid characterization or genomic analysis, could not be performed due to limited resources. Second, as this was a single-center study with a few resistant isolates, the findings cannot be generalized to the broader population. Third, due to the lack of clinical outcome data, the therapeutic implications of colistin resistance could not be assessed. Fourth, due to financial constraints, only 1% of colistin non-resistant isolates were tested for the detection of the *mcr-1* gene.

### CONCLUSION

Colistin resistance among clinical *E. coli* isolates in this setting was uncommon but clinically relevant. The detection of *mcr-1* in a subset of phenotypically resistant isolates highlights the importance of confirmatory susceptibility testing and ongoing molecular surveillance.

### Compliance with the Strengthening of the Reporting of Observational Studies in Epidemiology (STROBE)

The authors confirm that they adhered to the STROBE reporting guidelines during the manuscript preparation.

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**Author contributions:** AS: Contributed to the data collection, data compilation, clinical and laboratory data analysis and interpretation, and preparation of the first draft of the manuscript; AP: Design of the study, reviewing and editing of the manuscript; PS: Review of data and manuscript editing and correction; PJ: Supervision of laboratory work; TZ: Literature search. All authors have read and agreed to the published version of the manuscript.

**Ethical approval:** The research/study was approved by the Institutional Review Board at the University Ethics Committee Medical (UECM) of Swami Vivekanand Subharti University, Meerut, Uttar Pradesh with the reference number SMC/ UCEM/2023/695/314, dated September 25, 2023.

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given consent for clinical information to be reported in the journal. The patient understand that the patient's names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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