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Absence of Plasmid-Mediated *mcr* Genes among Phenotypically Colistin-Resistant Carbapenem-Resistant Gram-Negative Isolates from a Tertiary Care Centre in Central India

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ABSTRACT

Background: Colistin has re-emerged as a last-resort antimicrobial for treating infections caused by carbapenem-resistant Gram-negative bacteria (CR-GNB). The global discovery of plasmid-mediated mobilized colistin resistance (*mcr*) genes has raised serious concerns regarding the horizontal dissemination of colistin resistance. However, data on the molecular basis of colistin resistance in India remain limited.

Objectives: To detect plasmid-mediated *mcr-1* to *mcr-8* genes among phenotypically colistin-resistant carbapenem-resistant Gram-negative clinical isolates.

Materials and Methods: A total of 56 phenotypically colistin-resistant isolates identified by broth microdilution were subjected to polymerase chain reaction (PCR) for the detection of *mcr-1* to *mcr-8* genes using published primers and standardized protocols. Appropriate positive and negative controls were included.

Results: None of the 56 colistin-resistant isolates harbored *mcr-1* to *mcr-8* genes. These findings suggest that colistin resistance in the study isolates is predominantly mediated by chromosomal mechanisms rather than plasmid-borne resistance determinants.

Conclusion: The absence of *mcr* genes among phenotypically resistant isolates indicates a predominance of chromosomal resistance mechanisms in this region. Continuous molecular surveillance is essential to detect the future emergence of transferable colistin resistance and to inform antimicrobial stewardship strategies.

Keywords: Colistin resistance; *mcr* genes; Carbapenem-resistant organisms; PCR; Molecular epidemiology.

INTRODUCTION

Antimicrobial resistance (AMR) is a rapidly escalating global health crisis, threatening the effective management of bacterial infections. Among the most concerning developments is the rise of carbapenem-resistant Gram-negative bacteria (CR-GNB), including *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, which are associated with high morbidity and mortality rates[1].

The resurgence of colistin as a last-resort antibiotic has been driven by the limited availability of effective therapeutic alternatives for CR-GNB infections. Colistin exerts its bactericidal activity by binding to lipid A in the lipopolysaccharide (LPS) layer of Gram-negative bacteria, leading to membrane disruption and cell death[2]. Despite its effectiveness, increased clinical and agricultural use has resulted in the emergence of colistin resistance worldwide[3].

A major breakthrough in understanding colistin resistance occurred in 2015 with the discovery of the plasmid-mediated *mcr-1* gene in *Escherichia coli* isolates from China[4]. Since then, multiple *mcr* variants (*mcr-1* to *mcr-10*) have been identified globally, raising concerns about horizontal gene transfer and rapid dissemination of resistance across bacterial species[5]. Unlike chromosomal mutations, plasmid-borne *mcr* genes pose a greater public health threat due to their mobility and potential for widespread transmission.

In India, reports of *mcr*-mediated resistance remain sporadic, and several studies have demonstrated colistin resistance in the absence of detectable *mcr* genes[6–8]. These observations suggest that chromosomal mechanisms, such as mutations in regulatory systems (*pmrAB*, *phoPQ*, *mgrB*) and lipid A modification pathways, may play a dominant role[9]. Given the clinical importance of distinguishing plasmid-mediated from chromosomal resistance, this study aimed to investigate the molecular basis of colistin resistance by detecting *mcr-1* to *mcr-8* genes among phenotypically colistin-resistant carbapenem-resistant isolates from a tertiary care hospital in central India.

MATERIALS AND METHODS

Study Design and Isolate Selection

This molecular analysis was conducted as part of a hospital-based cross-sectional study in the Department of Microbiology, Index Medical College Hospital & Research Centre, Indore, from January 2022 to June 2025.

A total of 56 non-duplicate Gram-negative bacterial isolates demonstrating phenotypic resistance to colistin (MIC ≥4 µg/mL by broth microdilution) were included. These isolates were obtained from blood, urine, and pus samples of patients with clinically significant infections.

Bacterial Identification and Phenotypic Testing

Initial identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact automated system (bioMérieux, France). Colistin susceptibility was confirmed using the broth microdilution method in accordance with CLSI 2023 guidelines[10].

DNA Extraction

Genomic DNA was extracted from overnight bacterial cultures using a commercially available extraction kit, following the manufacturer's protocol. The quality and quantity of extracted DNA were assessed spectrophotometrically.

Polymerase Chain Reaction for *mcr* Genes

All isolates were screened for *mcr-1* to *mcr-8* genes using polymerase chain reaction (PCR). Published primer sequences and cycling conditions were employed for each target gene[11]. Each PCR run included known positive controls and nuclease-free water as a negative control.

Amplified products were analyzed by agarose gel electrophoresis and visualized under ultraviolet illumination after ethidium bromide staining.

RESULTS

All 56 phenotypically colistin-resistant carbapenem-resistant Gram-negative isolates were subjected to molecular screening. None of the isolates showed amplification of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, or *mcr-8* genes.

The absence of plasmid-mediated *mcr* genes across isolates from diverse clinical specimens suggests that colistin resistance in this study population is not driven by transferable resistance determinants.

DISCUSSION

The emergence of plasmid-mediated colistin resistance has generated global concern due to the potential for rapid horizontal dissemination of resistance genes across bacterial populations[4,5]. In this study, none of the phenotypically colistin-resistant isolates harbored *mcr* genes, indicating a predominance of chromosomal resistance mechanisms.

Similar findings have been reported from several Indian and international studies, where colistin resistance was observed in the absence of *mcr* genes[6–8,12]. Chromosomal mutations affecting regulatory pathways such as *pmrAB*, *phoPQ*, and *mgrB* have been implicated in lipid A modification, leading to reduced colistin binding[13].

The absence of *mcr* genes in this study is reassuring from a public health perspective, as plasmid-mediated resistance poses a greater threat due to its mobility. However, chromosomal resistance remains clinically significant and may still lead to treatment failure if undetected[14].

These findings underscore the importance of integrating molecular diagnostics with phenotypic testing. While routine *mcr* gene screening may not yet be warranted in all settings, periodic surveillance is essential to detect emerging plasmid-mediated resistance and to guide infection control policies[15].

CONCLUSION

This study demonstrates that phenotypic colistin resistance among carbapenem-resistant Gram-negative isolates in a tertiary care hospital in central India is not mediated by plasmid-borne *mcr-1* to *mcr-8* genes. The resistance is likely attributable to chromosomal mechanisms. Ongoing molecular surveillance, rational antimicrobial use, and robust stewardship programs are critical to preventing the emergence and spread of transferable colistin resistance.

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