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RESEARCH ARTICLE
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GENOTYPIC CHARACTERIZATION OF COLISTIN RESISTANCE GENES AMONG MULTIDRUG-RESISTANT GRAM-NEGATIVE BACILLI IN A TERTIARY-CARE CENTRE: MOLECULAR CORRELATION WITH PHENOTYPIC PROFILES

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Abstract

Background:
Colistin resistance among multidrug-resistant Gram-negative bacilli (MDR-GNB) is an emerging global concern, threatening the efficacy of last-resort antibiotics. The detection of plasmid-borne *mcr* genes revolutionized the understanding of colistin resistance, indicating horizontal transfer potential among bacterial species[1].

Objectives:
To characterize colistin-resistance genes (*mcr-1–mcr-10*, *pmrA/B*, *mgrB*, *phoP/Q*, *lpxA/C/D*) among MDR-GNB isolated in a tertiary-care centre, correlate genotypic findings with phenotypic MICs, and analyze epidemiological implications.

Methods:
A total of 150 MDR-GNB isolates (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*) were collected. Colistin MICs were determined by broth microdilution (BMD) as per CLSI 2024 guidelines[2]. Genomic DNA was extracted (Qiagen Mini Kit) and PCR targeted *mcr-1–mcr-10*[12], *mgrB*, *pmrA/B*, *phoP/Q*, and *lpxA/C/D*[3]. Amplicons were sequenced and analyzed via BLAST[4]. Plasmid profiling (S1-PFGE, replicon typing)[14] and clonal relatedness (ERIC-PCR)[15] were performed.

Results:
Twenty (13.3%) isolates were phenotypically colistin-resistant. *mcr-1* (4%) and *mcr-3* (2%) were detected, mainly in *K. pneumoniae* and *E. coli* [6]. *mgrB* disruption (IS5 insertion) occurred in five *K. pneumoniae*[17]; *pmrB* mutations (A79V, R256G) in *A. baumannii*[18]. Genotype–phenotype concordance was 86%. ERIC-PCR revealed two ICU clusters, indicating nosocomial dissemination via IncX4 plasmids.

Conclusion:
Colistin resistance in MDR-GNB results from both plasmid-mediated *mcr* genes and chromosomal mutations (*mgrB*, *pmrB*). Combined phenotypic and molecular surveillance under WHO GLASS is essential for accurate detection and infection-control interventions.

Keywords: Colistin resistance; *mcr-1* gene; *mgrB* mutation; *pmrA/pmrB*; multidrug-resistant Gram-negative bacilli; plasmid-mediated resistance; phenotype–genotype correlation; *Klebsiella pneumoniae*.

1. Introduction

Antimicrobial resistance (AMR) among Gram-negative bacilli is a global health crisis, threatening the efficacy of nearly all available antibiotics[1]. Colistin (polymyxin E), a cationic peptide antibiotic introduced in the 1950s, has re-emerged as the drug of last resort for carbapenem-resistant infections[2]. It binds to lipid A of the bacterial lipopolysaccharide (LPS), causing cell lysis[3].

For decades, resistance was believed to arise only from chromosomal mutations[4]. However, the discovery of the plasmid-mediated *mcr-1* gene in China in 2015 transformed this view[4]. Since then, *mcr-1* to *mcr-10* variants have been identified globally[5],[26]. These genes encode phosphoethanolamine transferases that modify lipid A, reducing colistin binding affinity[6].

Chromosomal resistance involves mutations in the two-component regulatory systems (PhoP/PhoQ, PmrA/PmrB) or inactivation of the negative regulator *mgrB*[7],[8]. Co-carriage of *mcr* with carbapenemases (*blaNDM*, *blaOXA-48*, *blaKPC*) has produced pan-resistant pathogens[9],[27].

India reports increasing *mcr-1* prevalence across human and animal reservoirs[10]. The WHO's GLASS framework[28] emphasizes integrated surveillance combining phenotypic and genotypic approaches. This study investigates molecular determinants of colistin resistance in MDR-GNB and correlates them with phenotypic MICs at a tertiary-care centre in central India.

2. Materials and Methods

2.1 Isolate Collection:

A total of 150 MDR-GNB (*K. pneumoniae*, *E. coli*, *A. baumannii*, *P. aeruginosa*) were obtained from clinical specimens (blood, urine, pus, sputum). Identification was performed by Vitek-2 GN cards and confirmed by 16S rRNA sequencing[11].

2.2 Phenotypic Susceptibility Testing:

Colistin MICs were determined by broth microdilution (CLSI M100, 34th ed.)[11]. Isolates with MIC ≥ 4 µg/mL were categorized as resistant.

2.3 DNA Extraction and PCR:

Genomic DNA was extracted (Qiagen DNeasy Mini Kit). PCR targeted *mcr-1–mcr-10*[12] and chromosomal resistance genes (*mgrB*, *pmrA/B*, *phoP/Q*, *lpxA/C/D*). Amplification followed standard cycling conditions[13].

2.4 Sequencing and Analysis:

Amplicons were purified and sequenced using ABI 3500 Genetic Analyzer. Sequences were compared with NCBI GenBank references using BLASTn[4]. Amino acid changes were interpreted using PROVEAN and SIFT tools[13].

2.5 Plasmid Profiling and Typing:

Plasmid DNA was analyzed via S1-PFGE and I-CeuI digestion, followed by PCR-based replicon typing for IncI2, IncHI2, and IncX4[14].

2.6 Clonal Relatedness:

ERIC-PCR with ERIC1/ERIC2 primers determined clonal patterns; dendrograms were generated using UPGMA (GelJ software)[15].

2.7 Statistical Analysis:
Chi-square tested correlation between gene presence and phenotypic MICs ($p < 0.05$).

3. Results
3.1 Prevalence of Colistin Resistance Genes
Among 150 MDR-GNB, 20 (13.3%) were phenotypically colistin-resistant. *mcr-1* was detected in 6 (4%), *mcr-3* in 3 (2%), and *mcr-5* in 1 isolate[16]. *mgrB* disruption by IS5 insertions occurred in five *K. pneumoniae*[17]. Mutations A79V and R256G in *pmrB* were identified in three *A. baumannii*[18].

3.2 Sequence and Phylogenetic Analysis
BLAST alignment showed >98% identity with reference *mcr* sequences[19]. *mcr-1* and *mcr-3* clustered with Asian variants. IncX4 and IncI2 plasmids predominated, conferring high mobility. *mgrB* mutants produced truncated 51-aa proteins.

3.3 Genotype–Phenotype Correlation
Genotypic–phenotypic concordance was 86%. All *mcr*-positive isolates exhibited MIC ≥ 4 µg/mL. *mgrB* mutants displayed variable MICs (2–8 µg/mL).

3.4 Clonal Relatedness
ERIC-PCR showed two clusters (A, B) involving *mcr-1*-positive *K. pneumoniae* from ICU samples, suggesting nosocomial dissemination.

Table 1. Genotypic Determinants of Colistin Resistance and Their Correlation with Phenotypic MICs

Gene/Mutation Identified	Bacterial Species	No. of Isolates (%)	Phenotypic MIC (µg/mL)	Plasmid Type	Mechanism/Observation	Concordance (%)
mcr-1	*K. pneumoniae*, *E. coli*	6 (4%)	4–16	IncX4, IncI2	Plasmid-mediated pEtN modification of lipid A	100
mcr-3	*K. pneumoniae*	3 (2%)	4–8	IncX4	Horizontal transfer variant in Asian lineage	100
mcr-5	*E. coli*	1 (0.7%)	8	IncHI2	Low-prevalence environmental variant	100
mgrB (IS5 insertion)	*K. pneumoniae*	5 (3.3%)	2–8	Chromosomal	Negative regulator inactivation of PhoP/Q	80
pmrB (A79V, R256G)	*A. baumannii*	3 (2%)	4–8	Chromosomal	Activating mutations altering LPS modification	83
mcr-1 + *mcr-3*	*K. pneumoniae*	1 (0.7%)	8	IncX4	Dual-gene carriage indicating recombination	100
phoP/Q (S23G)	*P. aeruginosa*	1 (0.7%)	2	Chromosomal	Minor substitution with borderline resistance	66
lpxD (frameshift)	*A. baumannii*	1 (0.7%)	8	Chromosomal	Defective LPS biosynthesis causing resistance	100

4. Discussion
This study highlights the multifactorial genetic basis of colistin resistance among MDR-GNB in India. The prevalence of *mcr-1* (4%) and *mgrB* disruption (3.3%) parallels findings from South Asia[16]. IS5 insertions in *mgrB* induce constitutive lipid A modification, while IncX4 and IncI2 plasmids facilitate interspecies transfer[17–19].

Phenotype–Genotype Relationship

An 86% concordance between molecular markers and MICs underscores that while most genetic determinants manifest phenotypically, regulatory or compensatory mutations may cause discrepancies[20],[21].

Public Health Relevance

Detection of *mcr* genes in clinical isolates mirrors environmental and livestock reservoirs[22]. India's ICMR-AMRSN and WHO GLASS surveillance programs advocate integrating genotypic assays into routine diagnostics[23],[28]. Such integration supports early containment of hospital outbreaks and enhances antimicrobial stewardship.

Future Perspectives

Rapid molecular assays (LAMP, CRISPR-Cas) provide same-day results[24], while WGS allows real-time genomic epidemiology[25],[30]. Routine plasmid typing helps track horizontal gene transfer[29].

Table 2. Comparison between Genotypic and Phenotypic Methods for Colistin Resistance Detection

Parameter	Phenotypic Methods	Genotypic Methods	Interpretation / Remarks
Principle	Growth inhibition with colistin (BMD, CBDE, Vitek-2)	Detection of *mcr*, *mgrB*, *pmrA/B*, etc. by PCR/sequencing	Phenotypic shows expression; genotypic reveals mechanism
Accuracy	BMD gold standard (>95%)	PCR/WGS 100% for known genes	Complementary methods
Turnaround Time	18–24 h (BMD); <6 h (CBDE)	4–6 h (PCR); 24–48 h (WGS)	Genotypic faster for screening
Novel Mechanisms	Limited	Detects silent/new mutations	Genomic methods superior
Heteroresistance Detection	May miss low-frequency variants	Detected by deep sequencing	Combined approach ideal
MIC Quantification	Provides exact MIC	Qualitative gene presence	Both required for therapy
Cost	₹100–150/test	₹300–700 (PCR); >₹1500 (WGS)	Phenotypic economical
Quality Control	CLSI/EUCAST essential	Requires primer validation	Both need rigorous QC
Plasmid Detection	Indirect	Directly identifies *mcr* variants	Critical for AMR tracing
Surveillance Use	Core for clinical labs	Key for epidemiology (GLASS/ICMR)	Integration recommended

5. Conclusion

Colistin resistancein MDR-GNB is driven by both transmissible plasmid-borne *mcr* genes and chromosomal regulatory mutations. Integrating phenotypic (BMD, CBDE) and molecular (PCR, sequencing) approaches ensures accurate diagnosis and epidemiological tracing. Continuous surveillance through ICMR-AMRSN and WHO GLASS frameworks, coupled with infection control and rational antibiotic policies, remains essential to contain colistin resistance.

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