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Colistin resistance and the *mcr–1* gene of *Pseudomonas aeruginosa*: Insights from Indian clinical isolates

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ABSTRACT

Objective: This study aimed to determine the prevalence of colistin resistance mediated by *mcr-1* in *Pseudomonas* (*P.*) *aeruginosa* isolates from clinical samples.

Methods: *P. aeruginosa* isolates were collected from various clinical specimens and tested for colistin resistance using the broth microdilution method. Isolates exhibiting resistance underwent polymerase chain reaction (PCR) screening for *mcr-1*. Antimicrobial susceptibility testing was performed for multiple antibiotics.

Results: Sixty five clinical isolates were obtained; 10 (15.4%) were phenotypically resistant to colistin, with minimum inhibitory concentrations (MIC) values ranging from 4-16 µg/mL. The *mcr-1* gene was detected in 4 (40.0%) colistin-resistant isolates, constituting 6.2% of all *P. aeruginosa* isolates. The highest resistance was observed for ceftazidime (70%), amikacin (60%), and ciprofloxacin (60%). Piperacillin-tazobactam was the most effective antibiotic, with 80% susceptibility.

Conclusions: The presence of *mcr-1* in *P. aeruginosa* isolates highlights the growing risk of colistin resistance beyond Enterobacteriaceae. Routine surveillance, antimicrobial stewardship, and molecular detection strategies are essential to curb further resistance spread.

KEYWORDS: *mcr-1* gene; Colistin resistance; *Pseudomonas aeruginosa*; Antimicrobial resistance; PCR detection

1. Introduction

The global rise of antimicrobial resistance constitutes a major threat to public health, particularly in the management of infections caused by multidrug-resistant Gram-negative bacteria. Among last-line treatment options, colistin has served as a critical therapeutic agent, especially against recalcitrant pathogens such as *Pseudomonas* (*P.*) *aeruginosa*. However, the clinical efficacy of colistin has been increasingly compromised by the emergence of mobilized colistin resistance (*mcr*) genes, most notably *mcr-1*[1,2]. First reported in China in 2015, *mcr-1* encodes a phosphoethanolamine transferase that modifies the lipid A moiety of lipopolysaccharide, reducing colistin affinity and enabling plasmid-mediated resistance. Since its discovery, *mcr-1* has been identified in a broad array of hosts and environments—including humans, animals, and ecological reservoirs—indicating its capacity for widespread horizontal gene transfer[3,4]. Although the majority of *mcr-1* reports have involved Enterobacteriaceae, occasional detection in *P. aeruginosa* is of particular concern. This pathogen is intrinsically adept at evading antimicrobial agents through a combination of chromosomal mutations, efflux systems, and biofilm formation. The potential convergence of these mechanisms with plasmid-borne *mcr* genes represents a formidable challenge for infection control and therapeutic interventions[5,6]. While both chromosomal and plasmid-driven resistance to polymyxins have been studied extensively[7,8], the molecular and clinical implications of *mcr–1* in *P. aeruginosa* remain inadequately characterized, especially in resource-limited healthcare settings. India, with its substantial antibiotic consumption and dense

Significance

Colistin resistance mediated by the *mcr-1* gene poses a major threat to clinical management of *Pseudomonas aeruginosa* in India, where antimicrobial resistance is rising. This study aims to define the prevalence, molecular mechanisms, and clinical impact of *mcr-1*-mediated resistance, offering insights into diagnostic improvements, therapeutic adaptations, and infection control policies. Findings will support antibiotic stewardship, guide surveillance strategies, and enhance patient management in resistant infections.

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clinical environments, presents a high-risk context for the emergence and propagation of resistant bacterial clones. The identification of *mcr-1*-positive *P. aeruginosa* in Indian clinical samples would mark a significant escalation in the resistance landscape, with serious consequences for both empirical treatment protocols and antimicrobial stewardship efforts. This study investigates the prevalence, resistance determinants, and genetic characteristics of *P. aeruginosa* strains harboring *mcr-1* in Indian hospital settings, aiming to inform public health strategies and enhance clinical preparedness.

2. Methods

2.1. Data collection

The study population comprised patients of all age groups presenting with clinically suspected bacterial infections at the hospital over the study period. Inclusion criteria involved all patients with clinical symptoms indicative of infection, from whom relevant clinical specimens (such as urine, blood, sputum, pus, wound swabs, and tracheal aspirates) were collected. Exclusion criteria included samples from patients currently receiving colistin therapy and those yielding contaminant or polymicrobial growth suggestive of colonization rather than infection.

2.2. Isolation and identification of P. aeruginosa

All collected specimens were processed using standard microbiological techniques. Culture media such as MacConkey agar and Cetrimide agar were used to facilitate selective growth. *P. aeruginosa* was presumptively identified on Cetrimide agar by its characteristic colony morphology—greenish pigment production and a grape-like odor, and was biochemically confirmed using a series of tests, including a positive oxidase test, a positive motility test, nitrate reduction, arginine dihydrolase activity, and growth at 42 °C.

2.3. Colistin susceptibility testing

Antimicrobial susceptibility to colistin was initially screened using the Broth Microdilution method, following the Clinical and Laboratory Standards Institute 2022 guidelines[9]. Isolates exhibiting resistance [minimum inhibitory concentration (MIC) > 2 µg/mL] were further confirmed using the Colistin Broth Disk Elution method to ensure accurate phenotypic detection. *Escherichia coli* ATCC 25922 served as the negative quality control strain for susceptibility testing.

2.4. Detection of mcr−1 gene

Confirmed colistin-resistant *P. aeruginosa* isolates were preserved in 15% glycerol stocks at -80 °C until further molecular testing. Plasmid DNA extraction was performed using a commercial plasmid mini-prep kit or alkaline lysis method, as per standard protocols. To detect the presence of the *mcr-1* gene, polymerase chain reaction (PCR) was carried out using primers specific for a 309 bp internal fragment (Forward: 5'-CGGTCAGTCCGTTTGTTC-3'; Reverse: 5'-CTTGGTCGGTCTGTAGGG-3'). PCR conditions followed the thermal cycling parameters described by Rebelo *et al*.,[10] which include initial denaturation, annealing, and extension phases optimized for *mcr-1* detection.

The amplified PCR products were subjected to agarose gel electrophoresis (1.5%) stained with ethidium bromide. Visualization was done under a UV transilluminator, and a 100 bp DNA ladder was used as a molecular weight marker. Samples producing a clear 309 bp band were considered positive for the *mcr-1* gene (Figure 1).

2.5. Ethical approval

This prospective study was conducted at the National Institute of Medical Sciences & Research, Jaipur, Rajasthan, following approval (NIMS/IEC/2021/0127) from the Institutional Ethics Committee. All procedures complied with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from all participants, and data were de-identified to protect patient privacy.

3. Results

3.1. Prevalence of colistin resistance and mcr−1 gene detection

A total of 65 *P. aeruginosa* isolates were included in this study. Among these 65 isolates, only 15.4% (10/65) were resistant to colistin by phenotypic MIC testing. As shown in Table 1, the range of colistin MIC for these isolates was 4 to 16 µg/mL. The majority of these colistin-resistant isolates were recovered from pus samples (60%, 6/10), with the remainder from endotracheal aspirates (20%, 2/10), blood (10%, 1/10), and urine (10%, 1/10).

Further molecular analysis was conducted on these 10 phenotypically resistant isolates to detect the *mcr−1* gene. The *mcr−1* gene was identified in 40% (4/10) of the colistin-resistant *P. aeruginosa* isolates. Although colistin-resistant isolates were from different clinical samples, the *mcr-1* gene was detected only from pus, blood and endotracheal aspirates isolates.

3.2. Co−resistance profile of colistin−resistant isolates

Colistin-resistant isolates were highly drug-resistant, as all of these pathogens were resistant to antibiotics like ceftadizime (70%), amikacin (60%), ciprofloxacin (60%), and tobramycin (40%). In contrast, the highest rate of sensitivity was noted only to piperacillin-tazobactam as 8 isolates (80%) were sensitive to this agent (Table 2).

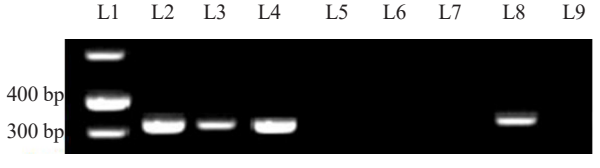


Figure 1. PCR products for *mcr*-1 gene detection. Lane 1: DNA ladder; Lanes 2,3,4, and 8: *mcr*-1-positive isolates; Lanes 5,6,7, and 9: Negative control.

Table 1. Characteristics of colistin-resistant *P. aeruginosa* isolates.

Specimens	Wards	MIC[†]	*mcr-1* gene
Urine	ICU[#]	16 μg/mL	Negative
Pus	Orthopedics	16 μg/mL	Negative
Pus	Orthopedics	16 μg/mL	Positive
Pus	Orthopedics	4 μg/mL	Negative
Pus	Pediatrics	16 μg/mL	Negative
Pus	Pediatrics	4 μg/mL	Negative
Pus	Medicine	4 μg/mL	Negative
Blood	ICU	8 μg/mL	Positive
Endotracheal aspirate	ICU	4 μg/mL	Positive
Endotracheal aspirate	ICU	4 μg/mL	Positive

[#]Intensive care unit; [†]Minimum inhibitory concentration.

Table 2. Antimicrobial susceptibility pattern of colistin resistant *P. aeruginosa isolates* (*n*=10).

Antimicrobials	Sensitive	Resistance
Tobramycin	6	4
Meropenem	7	3
Imipenem	7	3
Gentamicin	5	5
Amikacin	4	6
Ciprofloxacin	4	6
Aztronem	6	4
Piperacillin-tazobactam	8	2
Cefepime	6	4
Ceftadizime	3	7
Ticarcillin-clavulanic acid	6	4
Chloramphenicol	0	1
Nitrofurantoin	1	0
Fosfomycin	1	0

Note: Chloramphenicol is only used for blood samples, fosfomycin and nitrofurotoin are only used for urine samples.

4. Discussion

Antibiotic resistance has become a critical concern, particularly in hospital environments where multidrug-resistant pathogens are frequently encountered. This study confirms the presence of the *mcr*-1 gene in 6.2% (4/65) of *P. aeruginosa* isolates, highlighting the increasing threat of colistin resistance. Our findings are consistent with a recent study conducted in Western Maharashtra, which reported colistin-resistant isolates in tertiary care hospitals. The detection of the *mcr*-1 gene in colistin-resistant isolates further reinforces concerns about its emergence in non-Enterobacteriaceae pathogens[11].

Similarly, a study in South Africa that analyzed 114 clinical isolates found colistin resistance rates between 1.0% and 8.2%, aligning with our observed phenotypic resistance rates. However, their study did not detect *mcr-1* in *P. aeruginosa*, suggesting that alternative resistance mechanisms, such as mutations in *pmrAB* and *phoPQ*, may play a role[12]. In contrast, a study from Romania identified *mcr-1*-positive *Escherichia coli* isolates in clinical samples for the first time[13]. This underscores how the *mcr-1* gene, initially reported in *Escherichia coli*, is now being detected in non-fermenting Gram-negative bacteria, increasing the risk of untreatable infections.

The high occurrence of colistin resistance in ICU patients (40%) observed in this study is consistent with global reports. The ICU environment is recognized globally as a hotspot for the emergence of colistin resistance, driven by intense selective pressure from high antibiotic usage, critical illness, and invasive supportive care. Meta-analyses of ICU-acquired Gram-negative infections report colistin resistance rates averaging around 5%, rising from approximately 4% before 2015 to 6% during 2015–2019, with regional variations—highest in Europe (8.2%) and lower in Asia and the Americas[14]. A study from India also reported a higher prevalence of *mcr-1* in colistin-resistant isolates compared to general ward isolates, supporting the notion that ICU patients are at increased risk[15].

This study revealed high resistance rates to ceftazidime (70%), amikacin (60%), and ciprofloxacin (60%). Studies conducted in India found a similar trend, where *mcr-1*-positive isolates were often resistant to multiple β-lactams and aminoglycosides[16]. A comprehensive meta-analysis indicating approximately 12.7% prevalence of polymyxin-resistant Gram-negative bacteria across India, with 8.6% of these isolates harboring *mcr* genes—valuable for national epidemiological context[17].

Given the rising prevalence of colistin resistance, hospitals should incorporate PCR-based screening for *mcr-1* in routine microbiological testing. Some studies suggest additional antimicrobials may serve as viable alternatives for colistin-resistant infections, though further synergy testing is required[18]. The implementation of antibiotic stewardship programs is crucial in curbing irrational and excessive use of antimicrobials, thereby reducing selective pressure for resistance, and preserving the efficacy of last resort antibiotics such as colistin[19].

The study was also conducted within a limited timeframe, restricting the depth of exploration and potentially overlooking long-term trends. One of the primary constraints is the sample size, which may limit the generalizability of the findings to a broader population. Acknowledging these limitations provides context for the findings and highlights areas for future research.

5. Conclusions

This study highlights the emergence of *mcr-1*-mediated colistin resistance in *P. aeruginosa*, supporting global concerns that resistance is no longer restricted to Enterobacteriaceae. The high ICU prevalence and multidrug resistance patterns suggest that *P. aeruginosa* is evolving under strong selective pressures. Further genomic studies are necessary to investigate additional resistance determinants.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Gautam PK and Prajapati B conceptualized the study. Gautam PK collected the data, and Tiwari ON formatted the manuscript. All the authors agree the content of the manuscript.

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