DOI: https://doi.org/10.54393/fbt.v4i04.190

FUTURISTIC BIOTECHNOLOGY

https://fbtjournal.com/index.php/fbt
ISSN (E): 2959-0981, (P): 2959-0973
Volume 4, Issue 4 (Oct-Dec 2024)

Original Article

OPEN ACCESS

Comparative Evaluation of Phenotypic Assays for Detecting *mcr*-Mediated Colistin Resistance in *Acinetobacter baumannii*

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ARTICLE INFO

Keywords:
Mobile Colistin Resistance Genes, Minimum Inhibitory Concentration, Antimicrobial Susceptibility Testing, Colistin Resistance

How to Cite:
Raza, M., Khan, S. J., Ali, R., Masood, M. F., Jafri, L., Rani, R., Sadiq, S., Akhtar, N., & Jamil, B. (2024). Comparative Evaluation of Phenotypic Assays for Detecting *mcr*-Mediated Colistin Resistance in Acinetobacter baumannii: Phenotypic Assays for *mcr*-Mediated Colistin Resistance in Acinetobacter baumannii. Futuristic Biotechnology, 4(04), 68-74. https://doi.org/10.54393/fbt.v4i04.190

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Received Date: 9th September, 2024
Acceptance Date: 7th November, 2024
Published Date: 31st December, 2024

ABSTRACT

Acinetobacter baumannii that is resistant to colistin has proven to be a significant problem in neonatal intensive care units (NICUs), where neonates are highly susceptible to multidrug-resistant pathogens. The introduction of plasmid-mediated mobile colistin resistance (mcr) genes has added to the global panic, but the prevalence of these genes in neonatal isolates is limited in South Asia. **Objectives:** To establish the frequency of *mcr-1* to *mcr-5* genes in colistin-resistant *A. baumannii* isolates of NICU patients and to compare the phenotypic assay of colistin susceptibility to establish a dependable and affordable diagnostic tool. **Methods:** Sources of thirteen *A. baumannii* isolates were blood samples, urine samples, and respiratory samples of NICU patients. Colistin resistance in carbapenem-resistant isolates was determined by disc diffusion, broth macro-dilution, disc elution, colistin agar, and minimum inhibitory concentration (MIC). *Mcr* gene screening was done genotypically using PCR and gene-specific primers. **Results:** Eight (61.5) isolates had the *mcr-1* gene with no detection of *mcr-2* to *mcr-5*. Isolates were all colistin resistant, with MICs ranging from 16 -32 μg/ml. Colistin agar method gave similar results to the broth assays, and it was found that it is a viable and low-cost alternative to parallel screening. **Conclusions:** This research is the first reported case of *mcr-1*-mediated colistin resistance in clinical isolates of *A. baumannii* in a neonatal unit in Pakistan. Enhanced antimicrobial stewardship and resistance monitoring of neonatal care are needed. In resource-limited settings, the colistin agar method is suggested to be used as a routine laboratory screening.

INTRODUCTION

Acinetobacter baumannii is a gram-negative bacillus that is non-motile and aerobic, and has become one of the most feared nosocomial pathogens with the reputation of multidrug resistance (MDR) and resistance to eradication in hospitals. *A. baumannii* is a highly adaptable species to the environment, which has helped it to thrive in healthcare-associated infections [1]. It is closely linked with the occurrence of device-related infections, ventilator-associated pneumonia, bacteremia, urinary tract infections, and meningitis. The genotoxic acquisition of exogenous resistance genes in the pathogen, in addition to inherent resistance determinants, has contributed to its worldwide dissemination during hospital outbreaks. The spread of the opportunistic pathogen, especially in medical institutions of low and middle-income countries, is a major challenge to the survival of neonatal lives [2]. In the most recent update of the WHO Bacterial Priority Pathogens List, carbapenem-resistant *A. baumannii* (CRAB) has been designated with the critical priority, highlighting its severe nature as a threat to human health

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[3]. Antibiotics, even though they revolutionized medicine initially, have been slowed down by MDR bacteria, which has been an indication that the antibiotic era may soon come to an end. The diffusion of MDR *A. baumannii* all over the world has created a more vulnerable situation for healthcare systems. Medical devices that cause infections include ventilators, catheters, and other risk factors such as surgical operations, burns, long-term hospital stay, and immunosuppressive treatments [4]. Neonatal Intensive Care Units (NICUs) constitute a very delicate system, and newborn babies housed in them are very vulnerable to infections. Helped by these special units, a broadening of the multidrug-resistant opportunistic organisms, which in most cases are harmless to healthy people, is possible. MDR *A. baumannii* infections among neonates are linked to a high mortality rate, common use of invasive support (e.g., ventilation, vasoactive medications), and few treatment options. Neonatal sepsis due to *A. baumannii* may result in complications that need vasoactive treatment, mechanical ventilators, and poor survival outcomes [4]. Therefore, treatment opportunities are becoming limited, which is a major challenge to contemporary medicine. Colistin is a polymyxin E antibiotic, which has been regarded as a last-resort treatment of life-threatening MDR bacterial infection [5-7]. In the past, it was assumed that colistin resistance could only be caused by chromosomal mutations (e.g., pmrA/pmrB). The plasmid-mediated colistin resistance that is credited to the *mcr-1* gene and revamped the concept of colistin resistance [6, 7]. Up to the present day, ten mcr gene variants have been reported in various bacterial species [8]. At present, *A. baumannii* is treated with colistin when it occurs in neonates. Colistin resistance has become a current epidemic of increasing concern in the field of public health, and is particularly alarming in Pakistan. Recent research observed 7.3% resistance of 150 isolates from five hospitals, and another study reported that 75.6% of *A. baumannii* and 23.7% XDR in a tertiary care hospital [9, 10]. It is of urgent need to identify colistin resistance by rapid and cost-effective phenotypic approaches. Although the disc diffusion technique is not advised by CLSI and EUCAST [11] because the large colistin molecules are not well diffused, broth microdilution (BMD) and macrodilution techniques are both labour-intensive and technically challenging. Up to now, there is limited information regarding colistin resistance in *A. baumannii* isolates that inhabit Pakistani hospitals in the neonatal unit.

This study aims to focus on gathering neonatal A. baumannii isolates, determining the prevalence of *mcr* genes (*mcr-1* through *mcr*-5), and finding a consistent and fast way of testing colistin susceptibility and the determination of MIC by comparing agar- and broth-based tests.

METHODS

This cross-sectional laboratory-based study was conducted in the Department of Microbiology, BJ Micro Lab, Rawalpindi, Pakistan (March to August 2023). Clinical samples (blood, urine, and respiratory secretions) were collected from neonates admitted to the NICU of a tertiary hospital in Islamabad, Pakistan. Written informed consent was taken from all participants. Samples were obtained using aseptic techniques, transported within 2 h at 2–8°C, and processed immediately in the microbiology laboratory. Urine and respiratory specimens were grown on MacConkey and blood agar, and blood samples were put into automated blood culture bottles. Standard biochemical tests were used to identify colonies that suggested the presence of Acinetobacter, and the API-20E system (bioMérieux, France) was used to validate the results. Verified isolates of *A. baumannii* were kept at -20°C in a broth made with 20% glycerol. The study complied with the ethical standards and regulations of the Department of Life Sciences at Abasyn University's Islamabad Campus, as well as BJ Micro Lab's Gulzar Quaid, Rawalpindi. According to our institution's ethical guidelines and national regulations. The phenotypic assays were performed using different colistin concentrations to determine the resistance pattern of all bacterial strains. The appearance of turbidity in broth indicated colistin resistance Mueller Hinton (MH) agar (HiMedia, India) and MH broth (SBio, Singapore) were prepared with cationic adjustment by adding 2mg CaCl2 (Sigma, Germany) and 1mg MgCl2 (Sigma, Germany) in 100 mL media as per CLSI guidelines to prepare Cationic Adjusted Mueller Hinton Agar/Broth (CAMHA/CAMHB) [12]. The pH of the media was adjusted between 7.2-7.4. Various antibiotics, used in standard clinical practice and encompassing different antimicrobial groups were tested for susceptibility testing via disk diffusion assay as per CLSI version 2022. Only those bacterial samples were selected that were resistant to carbapenems [13]. Colistin stock solution (37.45 µg/mL colistin base equivalent) was serially diluted in CAMHB to obtain concentrations of 1–4 µg/mL. Tubes were inoculated with 10 µL of bacterial suspension (0.5 McFarland) and incubated at 37°C for 18–24 h. Turbidity indicated resistance. CLSI breakpoints were applied (S ≤ 2 µg/mL; R ≥ 4 µg/mL) [14]. *E. coli* ATCC 25922 (susceptible) and *E. coli* NCTC 13846 (*mcr-1* positive control) were included as reference strains to validate test accuracy. The disc elution method was performed by preparing 100 mL CAMHB. The inoculum was prepared in 0.5 mL of normal saline. The turbidity of this suspension was adjusted to 0.5 McFarland standards. As one colistin disc is of 10µg strength so

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colistin concentrations of 10- 40µg/mL were prepared by adding 1, 2, 3, and 4 colistin discs, respectively, in each test tube containing 1ml autoclaved distilled water, followed by incubation at room temperature for 30-40 min to elute disc components. Tubes were inoculated and incubated as above. Minimum inhibitory concentration (MIC) was assessed in broth and agar-based systems, using two-fold serial dilutions (4–128 µg/mL) in separate test tubes. The inoculum was prepared by dissolving a bacterial colony in normal saline, and 10 µL of the inoculum was added to each test tube. For the growth control, 1 mL of Mueller-Hinton (MH) broth was added to a test tube, followed by the addition of 10 µL of the inoculum. For the negative control, 1 mL of MH broth was added to a separate test tube without inoculum. The prepared test tubes were incubated at 37°C overnight. A growth control (inoculated CAMHB) and a negative control (uninoculated broth) were included for each assay [15]. Eluted colistin solution (prepared by dissolving colistin discs in autoclaved distilled water at room temperature for 30-40 min) was incorporated into CAMHA to prepare final concentrations of 2–32 µg/mL. Ten microliters of bacterial suspension were spotted onto plates and incubated at 37°C for 24 h. Growth indicated resistance. Parallel testing with *E. coli* NCTC 13846 and P. aeruginosa ATCC 27853 was performed for validation [16]. The DNA was extracted from all strains using the CTAB method with slight modifications. Briefly, a loop full of overnight-grown bacterial culture was taken into an Eppendorf containing freshly prepared CTAB lysis buffer (500 µL), proteinase K (10µL) (BioShape, Canada), 10% SDS (40µL) (Sigma Aldrich, Germany), and β-mercapto-ethanol (2 µL). The mixture was incubated at 950°C/2 hours, and finally, chloroform: isoamyl alcohol (500 0L) was added to the mixture, and centrifugation was done at 13000 rpm, and three layers were formed. A new Eppendorf tube was dried by vortexing it, and 20 mL of aqueous solution was transferred into it. Chilled isopropanol was added to the supernatant and incubated at room temperature. The pellet was centrifuged, washed with 70% ethanol, and dissolved in low TE buffer and kept at -20°C. On agarose gels stained with ethidium bromide, extracted DNA was stained [17]. Gradient PCR was used to verify the annealing temperature of each gene. The 25 µL of PCR reaction mixture was put together, and in it, 12.5 µL of master mix (Ab clonal), 2 µL of forward and reverse primers, 3.5 µL of PCR water, and 3-5 µL of template DNA were incorporated. The amplification conditions were: 25 cycles with initial denaturation at 94°C 10 min, denaturation at 94°C 30s, primer annealing at 51°C, 56°C, 57°C, 58°C and 58°C, respectively, extension at 72°C 60s, and final extension at 72 o C 10 min in theromocycler (Life-ECO). The presence of *mcr* genes was detected on a 1.5% agarose gel. *E. coli* NCTC 13846 (*mcr-1* positive) served as a positive control, while PCR-grade water was used as a negative control. To strengthen assay validation, at least one representative amplicon per target was purified and sequenced commercially to confirm gene identity via BLAST analysis. Descriptive statistics were used to summarize phenotypic and genotypic results. MIC values from broth and agar-based methods were compared using Spearman's rank correlation and Cohen's kappa (κ) for agreement analysis. Statistical analysis was done by SPSS version 27.0, and $p<0.05$ was considered significant.

RESULTS

Confirmed *A. baumannii* isolates were preserved in 20% glycerol broth at –20°C (Figure 1).

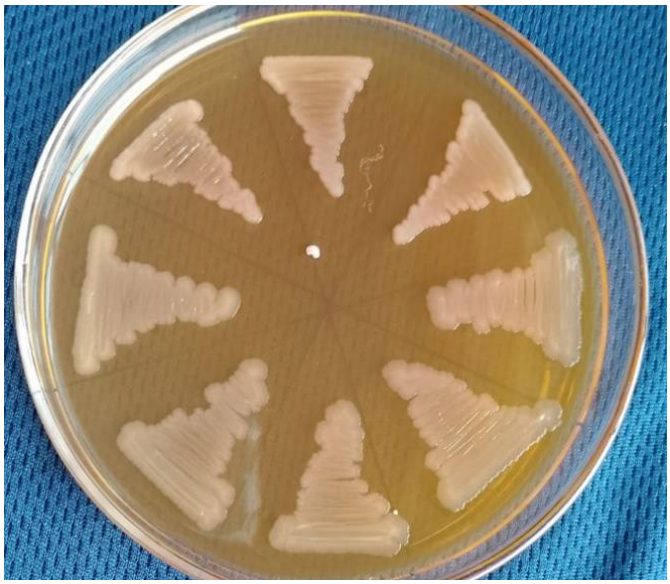

Figure 1: Growth of *A. baumannii* on MacConkey Agar

Extracted DNA was visualized on 1.5% agarose gels stained with ethidium bromide (Figure 2)

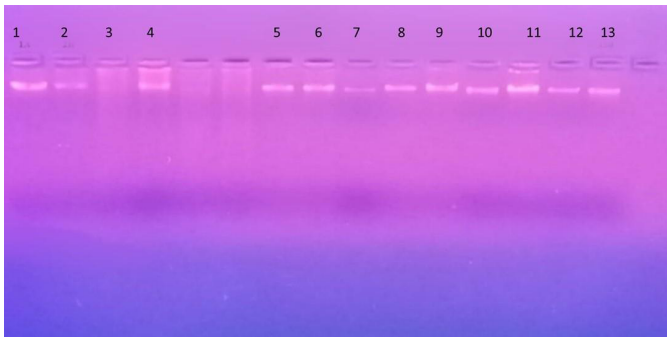

Figure 2: Agarose Gel Electrophoresis for Detection of Mcr Genes in *A. Baumannii* Isolates

The amplification of mcr1-5 genes was carried out by using the following sets of primers. Gene names, forward and reverse primer sequences, and their annealing temperature, expected product sizes, and annealing temperatures used for the amplification of mcr 1-5 genes in this study (Table 1).

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Table 1: The Amplification of *mcr1-5* Genes

Gene	Forward Primer	Reverse Primer	Product Size	Ta
mcr-1	AGTCCGTTTGTTCTTGTGGC3'	AGATCCTTGGTCTCGGCTTG	320 bp	51°C
mcr-2	CAAGTGTGTTGGTCGCAGTT3'	TCTAGCCCGACAAGCATACC	715 bp	56°C
mcr-3	AAATAAAAATTGTTCCGCTTATG	AATGGAGATCCCCGTTTTT	>929 bp	57°C
mcr-4	TCACTTTCATCACTGCGTTG	TTGGTCCATGACTACCAATG	1116 bp	57°C
mcr-5	ATGCGGTTGTCTGCATTTATC	TTGGTCCATGACTACCAATG	1644 bp	58°C

Confirmed *A. baumannii* isolates were preserved in 20% glycerol broth at –20°C (Figure 1).

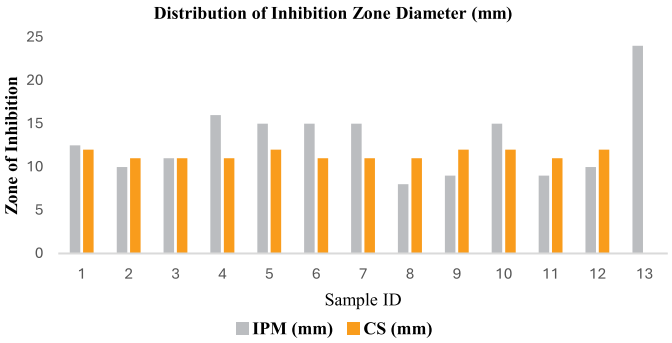

Figure 2: Zone of Inhibition Produced by *A. baumannii* Isolates

The recommended CLSI (2023) cutoff for colistin by broth macro-dilution is S ≤ 2 µg/mL and R ≥ 4 µg/mL. No statistical difference was observed in optical density among replicates ($p>0.05$), indicating consistent assay reproducibility. In the disc elution method, the results were comparable to the broth macro-dilution method; all strains showed resistance against colistin-eluted solutions at concentrations of 1, 2, 3, and 4 µg/mL. This consistency further validated the reliability of broth-based methods over disc diffusion for detecting colistin resistance. For MIC determination, two methods were compared colistin agar test (CAT) and the BMD. Comparable results were obtained using both methods. All strains showed resistance to colistin at concentrations ≥ 4 µg/mL. Results show high-level resistance across all isolates (MIC ≥ 16 µg/mL)(Figure 3).

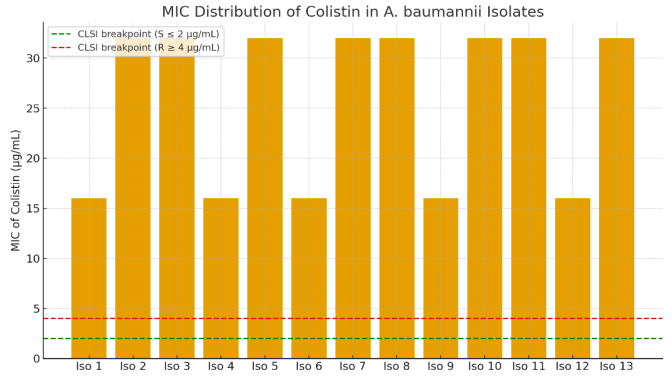

Figure 3: MIC of Colistin against *A. baumannii* Isolates in CAMH Broth

These results follow the colistin agar assay, where: (a) 8 µg/mL showed growth of all isolates; (b) 16 µg/mL showed inhibited growth in strains 1, 7, and 9; and (c) 32 µg/mL showed complete growth inhibition. Scale bar = 20 mm (Figure 4).

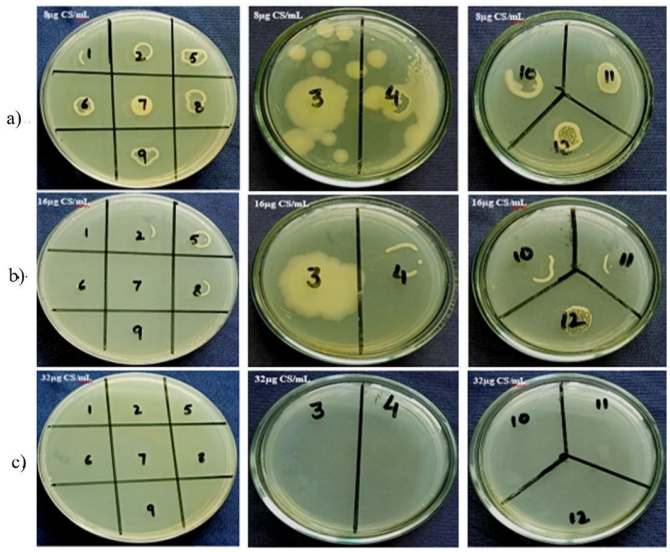

Figure 4: Evaluation of Colistin Resistance on Agar Plates Containing Different Colistin Concentrations

All the colistin-resistant strains of *A. baumannii* were screened for the presence of mcr1 to 5 genes. Only the *mcr-1* gene was detected in strains 1, 2, 3, 5, 6, 7, 8, and 9; however, other resistance determinants, including *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5*, were not found in any of the strains. Lane M: 100 bp DNA ladder; Lanes 1–9: *A. baumannii* isolates; Lane C+: positive control; Lane N: negative control. Bands confirmed the presence of mcr-1 in eight isolates (1, 2, 3, 5, 6, 7, 8, and 9) (Figure 5).

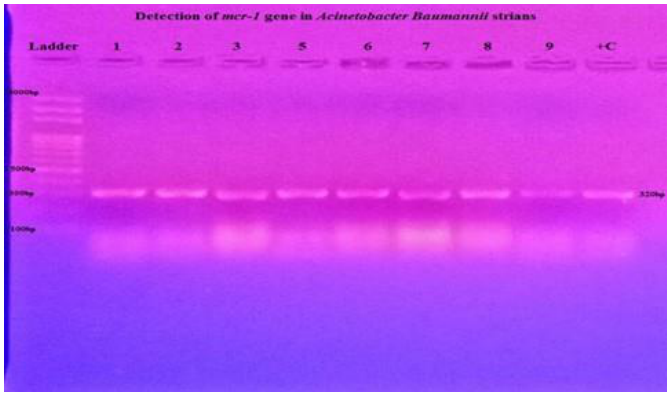

Figure 5: Visualization of PCR-Amplified *mcr-1* Gene (320 bp) Bands on 1.5% Agarose Gel Electrophoresis

DISCUSSION

One of the most widely spread mcr determinants, the *mcr-1* gene, has been reported in different bacterial strains in more than 60 countries [18, 19]. This paper presents the initial findings of the *mcr-1* gene in *A. baumannii* isolates in the NICU of a tertiary hospital in Islamabad, Pakistan. The

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study found that 8 of 13 (61.5) of the isolates had genotypic resistance to colistin since they had *mcr-1*, and the rest (38.5) of the isolates were resistant to colistin, but that could be due to other mcr variants (like *mcr-6* to *mcr-10*) or could be because of chromosomal modes of resistance. Such mechanisms are the lipid A modification through the addition of L-phosphoethanolamine (PEtN) or 4-amino-4-deoxy-L-arabinose (L-Ara4N), impairment of lipopolysaccharide biosynthesis genes, or expression of efflux pumps [20, 21]. On the contrary, 37 carbapenem-resistant *A. baumannii* (CRAB) isolates were analyzed by Germ *et al.* who detected no *mcr-1* to *mcr-5* gene [22]. In our investigation, all isolates showed an inhibition zone that did not exceed 12 mm in the disc diffusion test. Nevertheless, another study in a similar manner has stated that the disc diffusion technique is not very reliable in detecting colistin resistance because of poor diffusion in agar media [23]. There was a comparative analysis of phenotypic assays performed in this research that showed significant methodological understanding. The five methods tested included disc diffusion, broth macro-dilution, disc elution, MIC on colistin agar, and broth MIC broth and agar-based MIC assays; all had the highest concordance with genotypic results (100%), although disc diffusion demonstrated low agreement. Both disc elution and broth macro-dilution assays were consistent in their categorical interpretation, meaning high reliability and diagnostic accuracy. Phenotypic approaches that have been validated in terms of their performance are still necessary where there is no molecular assay, especially in low-resource laboratories. The research establishes colistin resistance in most of the isolates through phenotypic and genotypic methods, and the use of reliability in in-house methods is crucial. The colistin agar technique turned out to be reproducible as well as practical, and less equipment is required; parallel testing can be done, which is an advantage to NICU surveillance in resource-constrained hospitals. The finding of *mcr-1*-positive isolates in a neonatal facility is also very disturbing, because neonates are extremely sensitive to blood-borne infections and they do not have many treatment options. Reports of this nature demonstrate the need to improve infection control measures that include hand hygiene, decontamination of reusable equipment, and frequent monitoring of antimicrobial resistance [24]. Our findings underscore the urgent need for antimicrobial stewardship in Pakistan's healthcare system, particularly in NICUs, where horizontal gene transfer and clonal spread of mcr-mediated resistance can occur rapidly. The study also reinforces the importance of continuous surveillance of *A. baumannii* and judicious use of colistin in both clinical and veterinary contexts. Although the sample size (n=13) was limited, it represented all carbapenem-resistant *A. baumannii* isolates collected during a six-month NICU surveillance period. This exploratory dataset provides baseline evidence to guide larger multicenter validation studies and the development of cost-effective diagnostic frameworks for colistin resistance monitoring in Pakistan.

CONCLUSIONS

This study provides the first evidence of *mcr-1*-mediated colistin resistance in *A. baumannii* from a Pakistani NICU. Colistin agar and broth-based MIC assays were the most reliable phenotypic tests, while disc diffusion performed poorly. Findings underscore the need for low-cost, validated diagnostics, routine molecular surveillance, and strengthened infection control. Despite a small sample size, the study highlights the urgent need for careful colistin use in clinical and veterinary settings.

Authors Contribution

Conceptualization: LJ, RR, BJ
Methodology: MR, MFM, RR, NA
Formal analysis: RA, SS
Writing review and editing: MR, SJK, RA, SS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Weinberg SE, Villedieu A, Bagdasarian N, Karah N, Teare L, Elamin WF. Control and Management of Multidrug-Resistant Acinetobacter Baumannii: A Review of the Evidence and Proposal of Novel Approaches. Infection Prevention in Practice. 2020 Sep; 2(3): 100077. doi: 10.1016/j.infpip.2020.100077.

[2] Arhoune B, Oumokhtar B, Hmami F, El Fakir S, Moutaouakkil K, Chami F *et al.* Intestinal Carriage of Antibiotic-Resistant Acinetobacter Baumannii Among Newborns Hospitalized in Moroccan Neonatal Intensive Care Unit. PLOS One. 2019 Jan; 14(1): e0209425. doi: 10.1371/journal.pone.0209425.

[3] Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E *et al.* The WHO Bacterial Priority Pathogens List 2024: A Prioritisation Study to Guide Research, Development, And Public Health Strategies Against Antimicrobial Resistance. The Lancet Infectious Diseases. 2025 Apr. doi: 10.1016/S1473-3099(25)00118-5.

DOI: https://doi.org/10.54393/fbt.v4i04.190

[4] Mahich S, Angurana SK, Suthar R, Sundaram V, Munda VS, Gautam V. Acinetobacter Sepsis Among Out-Born Neonates Admitted to Neonatal Unit in Pediatric Emergency of A Tertiary Care Hospital in North India. The Indian Journal of Pediatrics. 2021 Feb; 88(2): 127-33. doi: 10.1007/s12098-020-03460-3.

[5] Torres RT, Cunha MV, Araujo D, Ferreira H, Fonseca C, Palmeira JD. Emergence of Colistin Resistance Genes (Mcr-1) in Escherichia Coli among Widely Distributed Wild Ungulates. Environmental Pollution. 2021 Dec; 291: 118136. doi: 10.1016/j.envpol.2021.118136.

[6] Al-Kadmy IM, Ibrahim SA, Al-Saryi N, Aziz SN, Besinis A, Hetta HF. Prevalence of Genes Involved in Colistin Resistance in Acinetobacter Baumannii: First Report from Iraq. Microbial Drug Resistance. 2020 Jun; 26(6): 616-22. doi: 10.1089/mdr.2019.0243.

[7] Hoang HT, Higashi A, Yamaguchi T, Kawahara R, Calvopina M, Bastidas-Caldés A *et al.* Fusion Plasmid Carrying the Colistin Resistance Gene Mcr of Escherichia Coli Isolated from Healthy Residents. Journal of Global Antimicrobial Resistance. 2022 Sep; 30: 152-4. doi: 10.1016/j.jgar.2022.06.007.

[8] Hussein NH, Al-Kadmy IM, Taha BM, Hussein JD. Mobilized Colistin Resistance (mcr) Genes from 1 to 10: A Comprehensive Review. Molecular Biology Reports. 2021 Mar; 48(3): 2897-907. doi: 10.1007/s11033-021-06307-y.

[9] Jabeen F, Khan Z, Sohail M, Tahir A, Tipu I, Saleem HG. Antibiotic Resistance Pattern of Acinetobacter Baumannii Isolated from Bacteremia Patients in Pakistan. Journal of Ayub Medical College Abbottabad. 2022 Jan; 34(1): 95-100. doi: 10.55519/JAMC-01-9105.

[10] Ahsan U, Mushtaq F, Saleem S, Malik A, Sarfaraz H, Shahzad M *et al.* Emergence of High Colistin Resistance in Carbapenem-Resistant Acinetobacter Baumannii in Pakistain and Its Potential Management Through Immunomodulatory Effect of an Extract from Saussurea lappa. Frontiers in Pharmacology. 2022 Sep; 13: 986802. doi: 10.3389/fphar.2022.986802.

[11] Matuschek E, Brolund A, Lindsjö OK, Giske CG, Byfors S, Kahlmeter G. Revisiting Colistin Susceptibility Testing: Will Adding Calcium to Mueller–Hinton Agar Improve the Detection of Colistin Resistance? Clinical Microbiology and Infection. 2021 Aug; 27(8): 1172-e1. doi: 10.1016/j.cmi.2021.04.014.

[12] Főldes A, Székely E, Voidăzan ST, Dobreanu M. Comparison of Six Phenotypic Assays with Reference Methods for Assessing Colistin Resistance in Clinical Isolates of Carbapenemase-Producing Enterobacterales: Challenges and Opportunities. Antibiotics. 2022 Mar; 11(3): 377. doi: 10.3390/antibiotics11030377.

[13] Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100. Journal of Clinical Microbiology. 2021 Nov; 59(12): 10-128. doi: 10.1128/JCM.00213-21.

[14] Weinstein MP. Performance Standards for Antimicrobial Susceptibility Testing. (No Title). 2018.

[15] Simner PJ, Bergman Y, Trejo M, Roberts AA, Marayan R, Tekle T *et al.* Two-Site Evaluation of the Colistin Broth Disk Elution Test to Determine Colistin in Vitro Activity Against Gram-Negative Bacilli. Journal of Clinical Microbiology. 2019 Feb; 57(2): 10-128. doi: 10.1128/JCM.01163-18.

[16] Turlej-Rogacka A, Xavier BB, Janssens L, Lammens C, Zarkotou O, Pournaras S *et al.* Evaluation of Colistin Stability in Agar and Comparison of Four Methods for MIC Testing of Colistin. European Journal of Clinical Microbiology and Infectious Diseases. 2018 Feb; 37(2): 345-53. doi: 10.1007/s10096-017-3140-3.

[17] Uwizeyimana JD, Kim D, Lee H, Byun JH, Yong D. Determination of Colistin Resistance by Simple Disk Diffusion Test Using Modified Mueller-Hinton agar. Annals of Laboratory Medicine. 2020 Jul; 40(4):306-11. doi: 10.3343/alm.2020.40.4.306.

[18] Cherak Z, Loucif L, Moussi A, Rolain JM. Epidemiology of Mobile Colistin Resistance (MCR) Genes in Aquatic Environments. Journal of Global Antimicrobial Resistance. 2021 Dec; 27: 51-62. doi: 10.1016/j.jgar.2021.07.021.

[19] Ling Z, Yin W, Shen Z, Wang Y, Shen J, Walsh TR. Epidemiology of Mobile Colistin Resistance Genes mcr-1 to mcr-9. Journal of Antimicrobial Chemotherapy. 2020 Nov; 75(11): 3087-95. doi: 10.1093/jac/dkaa205.

[20] Talebi Bezmin Abadi A, Rizvanov AA, Haertlé T, Blatt NL. World Health Organization Report: Current Crisis of Antibiotic Resistance. Bio-Nano Science. 2019 Dec; 9(4): 778-88. doi: 10.1007/s12668-019-00658-4.

[21] Novović K and Jovčić B. Colistin Resistance in Acinetobacter Baumannii: Molecular Mechanisms and Epidemiology. Antibiotics. 2023 Mar; 12(3): 516. doi: 10.3390/antibiotics12030516.

[22] Germ J, Poirel L, Kisek TC, Spik VC, Seme K, Premru MM *et al.* Evaluation of Resazurin-Based Rapid Test to Detect Colistin Resistance in Acinetobacter Baumannii Isolates. European Journal of Clinical Microbiology and Infectious Diseases. 2019 Nov; 38(11): 2159-62. doi: 10.1007/s10096-019-03657-1.

DOI: https://doi.org/10.54393/fbt.v4i04.190

[23] Thadtapong N, Chaturongakul S, Soodvilai S, Dubbs P. Colistin and Carbapenem-Resistant Acinetobacter baumannii Aci46 in Thailand: Genome Analysis and Antibiotic Resistance Profiling. Antibiotics. 2021 Aug; 10(9): 1054. doi: 10.3390/antibiotics10091054.

[24] Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM *et al.* Multiplex PCR for Detection of Plasmid-Mediated Colistin Resistance Determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for Surveillance Purposes. Eurosurveillance. 2018 Feb; 23(6): 17-00672. doi: 10.2807/1560-7917.ES.2018.23.6.17-00672.