International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 14, No. 3, March 2025 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_14.3.2025.217

Original Research

Comparative Evaluation Of Colistin Broth Disc Elution Method, And Broth Microdilution Method To Detect Invitro Action Of Colistin Against Carbapenemase-Producing Enterobacteriaceae

Dr. Swathy.S.R[1], Dr. Vijaya Swetha V, MD[2], Dr. A. Ankamma, MD[3], Dr. N. Padma Priya, MD[4]

[1]Final Year Post Graduate, Department of Microbiology, Government Medical College, Ongole, Andhra Pradesh, India
[2]Assistant Professor, Department of Microbiology, Government Medical College, Ongole, Andhra Pradesh, India.
[3]Associate Professor, Department of Microbiology, Government Medical College, Ongole, Andhra Pradesh, India.
[4]Professor and HOD, Department of Microbiology, Government Medical College, Ongole, Andhra Pradesh, India.

Corresponding Author
Dr. Vijaya Swetha V
Assistant Professor, Department of Microbiology, Government Medical College, Ongole, Andhra Pradesh, India.
Email ID: – vuppalapati18@gmail.com

Received date: 29 February 2025 Acceptance date: 30 March 2025

ABSTRACT

Background: The rise of Carbapenemase-Producing Enterobacteriaceae (CPE) presents a critical challenge to global public health, with colistin serving as one of the few remaining therapeutic options. Accurate determination of colistin Minimum Inhibitory Concentration (MIC) is essential to ensure appropriate clinical use. While Broth Microdilution (BMD) is the gold standard for MIC determination, it is often impractical in resource-limited settings. The Colistin Broth Disc Elution (CBDE) method has emerged as a simpler, cost-effective alternative.

Aim: To compare the efficacy of the Colistin Broth Disc Elution method with the Broth Microdilution method in determining colistin MIC in Carbapenemase-Producing Enterobacteriaceae.

Materials and Methods: A prospective study was conducted from August 2024 to January 2025 at Government Medical College, Ongole, Andhra Pradesh. A total of 400 clinical samples were collected and processed using standard microbiological techniques. Carbapenemase production in Enterobacteriaceae was confirmed using the Modified Carbapenem Inactivation Method (mCIM). Colistin susceptibility was tested using both CBDE and BMD methods according to CLSI and EUCAST guidelines. Categorical Agreement (CA) and Essential Agreement (EA) were calculated for comparative analysis.

Results: Out of 400 samples, 43 CPE isolates were identified. BMD showed MICs ranging from 0.25 to 8 µg/mL, while CBDE showed MICs from ≤1 to ≥4 µg/mL. CBDE demonstrated a categorical agreement of 100% and essential agreement of 97.67% with BMD.

Conclusion: The CBDE method shows excellent concordance with BMD, making it a reliable and accessible alternative for colistin MIC determination, especially in low-resource settings.

Keywords: Colistin, Carbapenemase-Producing Enterobacteriaceae, Broth Microdilution, Broth Disc Elution, Antimicrobial Resistance, MIC Testing.

INTRODUCTION

The emergence and spread of Carbapenemase-producing Gram-negative bacteria (CPGNB) have become a major threat to public health due to their resistance to a broad range of antibiotics. These organisms, particularly those within the Enterobacteriaceae family, produce carbapenemases that neutralize the efficacy of carbapenem antibiotics, which were once considered the cornerstone of treatment for multidrug-resistant (MDR) infections [5]. As a result, the medical community has turned to colistin, a polymyxin antibiotic, as a last-resort option

DOI: 10.69605/ijlbpr_14.3.2025.217

to treat infections caused by carbapenem-resistant bacteria. Despite its associated risks, including nephrotoxicity and neurotoxicity, colistin remains a vital treatment for severe infections [1,2]. However, the widespread and prolonged use of colistin has led to the development of resistance, compounding the difficulties of managing infections and contributing to higher rates of morbidity and mortality [4]. Colistin resistance arises through genetic mutations that alter the bacterial cell membrane or via the acquisition of plasmid-mediated resistance factors like the mcr-1 gene, further limiting treatment options [6,7]

Determining the minimum inhibitory concentration (MIC) of colistin is crucial for effective clinical management of infections. MIC testing helps to choose appropriate dosage and prevents the misuse of colistin, which could exacerbate resistance. The Broth Microdilution method is widely regarded as the gold standard for colistin MIC testing and is endorsed by international organizations like EUCAST and CLSI [8]. However, this method requires specialized laboratory equipment and expertise, which can make it impractical in many healthcare settings, particularly in low-resource environments [7]

Given these challenges, alternative methods for colistin MIC testing have been explored. One promising option is the Colistin Broth Disc Elution (CBDE) method, which has shown potential in providing reliable results while being simpler and more cost-effective than traditional methods. The CBDE method has demonstrated its ability to accurately assess colistin susceptibility in Gram-negative bacteria, offering a viable option for facilities that lack access to advanced laboratory tools [1,2]. By expanding the use of such accessible methods, it may be possible to improve the monitoring of colistin resistance and enhance antimicrobial resistance surveillance efforts globally.

This study seeks to evaluate the effectiveness and reliability of the CBDE method as an alternative to the Broth Microdilution technique for determining colistin MIC, particularly in the context of infections caused by carbapenem-resistant Gram-negative bacteria.

AIM

To perform a comparative evaluation of the Colistin Broth Disc Elution method and the Broth Microdilution method against carbapenemase producing Enterobacteriaceae.

OBJECTIVES

To isolate Carbapenemase Producing Enterobacteriaceae.

To determine MIC of Colistin in Carbapenemase-Producing Enterobacteriaceae by Colistin Broth Disc Elution method and Broth Microdilution test.

To compare the efficacy of the Colistin Broth Disc Elution method to the Broth Microdilution method to determine the MIC of Colistin.

MATERIALS & METHODS

Study Design: A prospective study was conducted.

Study Location: The study was performed at the Department of Microbiology, Government Medical College, Ongole, Andhra Pradesh.

Study Duration: The study duration was from August 2024 to January 2025.

Sample Size and Types

A total of 400 clinical samples, including urine, exudate, sputum, and blood, were collected. The samples were selected using simple random sampling[10],[11], calculated with a prevalence (p) of 50.0106%, q = 100 - p, and an absolute precision (L) of 10%.

Inclusion Criteria

Patients of all age groups and both sexes.
Patients who provided informed and written consent.

Exclusion Criteria

Isolates other than Enterobacteriaceae.
Patients who did not provide written consent.

Sample Collection and Processing

Sample processing: Samples were collected under aseptic conditions and processed following standard microbiological protocols. Among the isolated Enterobacteriaceae, carbapenemase production was detected using the Modified Carbapenem Inhibition Method (mCIM)[9]. The activity of colistin was evaluated using the Colistin Broth Disc Elution (CBDE) method[12] and the Colistin Broth Microdilution method [11], adhering to CLSI and EUCAST guidelines for carbapenemase-producing Enterobacteriaceae, and a comparative evaluation of the results from both methods was done.

Modified Carbapenem Inactivation Method (mCIM)

A loopful of bacterial isolate grown overnight on agar was inoculated into 2 mL of trypticase soy broth (TSB) and vortexed. A 10 µg meropenem disk was immersed in the broth and incubated at 35°C for 4 hours. Concurrently, a 0.5 McFarland suspension of E. coli ATCC 25922 was prepared and used to inoculate Mueller-Hinton Agar (MHA). After incubation, the meropenem disk was carefully removed from the broth, excess liquid removed, and placed onto the inoculated MHA plate. Plates were incubated overnight at 35°C. Carbapenemase production was interpreted as positive with zones of inhibition measuring 6–15 mm or growth within a 16–18 mm zone, and negative with zones of inhibition ≥19 mm.

Colistin Broth Disc Elution (CBDE) Method

For each isolate tested, four glass tubes labeled Control, 1, 2, and 4 were prepared with 10 mL of cation-adjusted Mueller-Hinton broth (CAMHB).

DOI: 10.69605/ijlbpr_14.3.2025.217

Colistin discs (10 µg each) were added to achieve final concentrations of 0 µg/mL (Control), 1 µg/mL (tube 1), 2 µg/mL (tube 2), and 4 µg/mL (tube 4). After incubation at room temperature for 30-60 minutes to allow colistin elution, discs were discarded. The broth was aliquoted into 1 mL volumes and stored at -20°C. Before testing, aliquots were thawed to room temperature, inoculated with 5 µL of standardized bacterial suspension (~7.5×10^5 CFU/mL), and incubated at 35 ± 2°C for 18–20 hours. Tubes were examined for turbidity, with MIC defined as the lowest colistin concentration inhibiting visible growth. Susceptibility interpretation was as follows:
Colistin Susceptible: MIC ≤2 µg/mL
Colistin Resistant: MIC ≥4 µg/mL

Colistin Broth Microdilution Method

The broth microdilution method utilized the Colistin HiMIC Plate Kit. A bacterial suspension equivalent to 0.5 McFarland standard was diluted twice to approximately 10^6 CFU/mL. From this, 200 µL was transferred into a HiMIC diluent tube, vortexed, and then 220 µL was inoculated into each of the eight wells labeled A to H on the kit. The strip was incubated at 35–37°C for 18 hours. Wells were assessed for color changes (purple to pink), indicating bacterial growth. MIC was determined as the lowest concentration without color change (remaining purple), interpreted as:
Colistin Susceptible: MIC ≤2 µg/mL
Colistin Resistant: MIC ≥2 µg/mL
A comparative evaluation between the CBDE method and broth microdilution method results was subsequently conducted.

RESULTS

Out of 400 samples collected, 251 (62.75%) were culture-positive, while 149 (37.25%) were sterile. Of the positive samples, 144 (57.37%) were Gram-negative bacilli (GNB) and 107 (42.63%) were Gram-positive cocci (GPC). Out of the 144 GNB isolates, 120 (83.33%) were Enterobacteriaceae, while 24 (16.67%) were non-Enterobacteriaceae. Among the 120 Enterobacteriaceae, 43 (35.83%) were carbapenemase-producing Enterobacteriaceae (CPE), with the remaining 77 (64.17%) being non-CPE. Colistin MIC testing of the 43 CPE isolates using broth microdilution (BMD) showed that 30 (69.77%) had an MIC of 0.25 µg/mL, 10 (23.26%) had 0.5 µg/mL, 2 (4.65%) had 1 µg/mL, and 1 (2.33%) had 8 µg/mL. The colistin broth disc elution (CBDE) method indicated that 33 isolates (76.74%) had a MIC ≤1 µg/mL, 9 (20.93%) had 2 µg/mL, and 1 (2.33%) had ≥4 µg/mL. The Colistin Broth Disk Elution (CBDE) method demonstrated strong concordance with the Broth Microdilution (BMD) method, achieving a categorical agreement (CA) of 100% and an essential agreement (EA) of 97.67%.

Table: 1. MIC estimated by Broth Microdilution (BMD) method

MIC in µg/mL	0.25	0.5	1	2	4	8
No. of isolates	30	10	2	0	0	1
%	69.77	23.26	4.65	0	0	2.33

Table: 2. MIC estimated by Colistin Broth Disc Elution (CBDE) method

MIC in µg/mL	≤1	2	≥4
No. of isolates	33	9	1
%	76.74	20.93	2.33

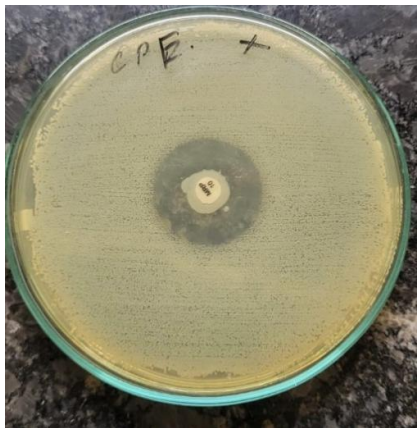

Fig.1 mCIM method –The isolate shown is a carbapenemase-producing Enterobacteriaceae

DOI: 10.69605/ijlbpr_14.3.2025.217

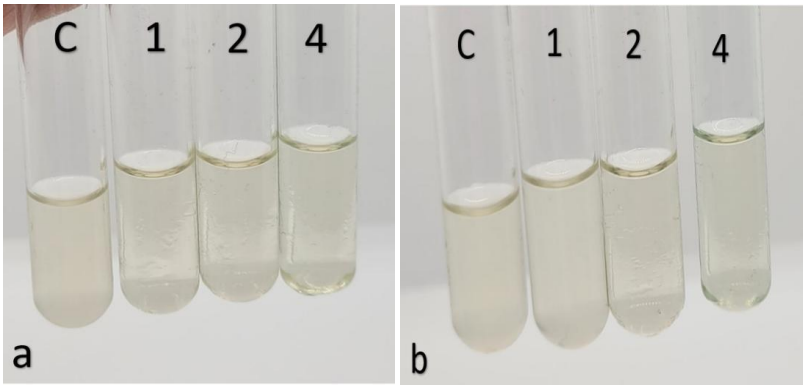

Fig.2 a) MIC by CBDE ≤1µg/mL, b) MIC by CBDE =2µg/mL

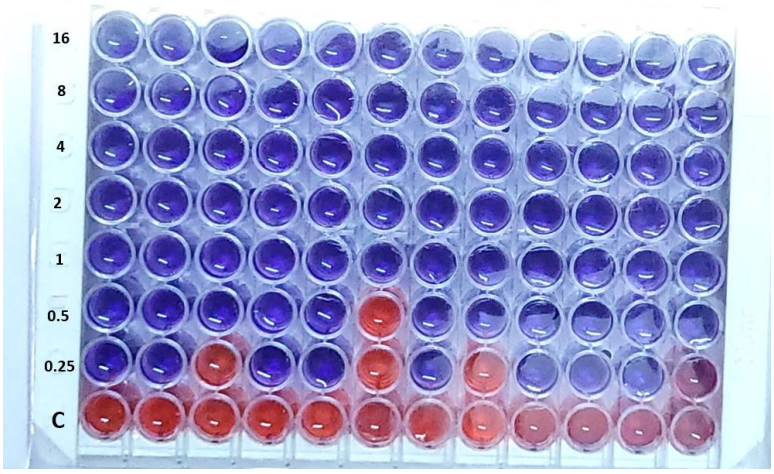

Fig.3 MIC by Broth Microdilution method using HiMIC kit. Pink indicates uninhibited growth, while purple signifies inhibition or absence of growth. The first purple well at the lowest concentration determines the MIC.

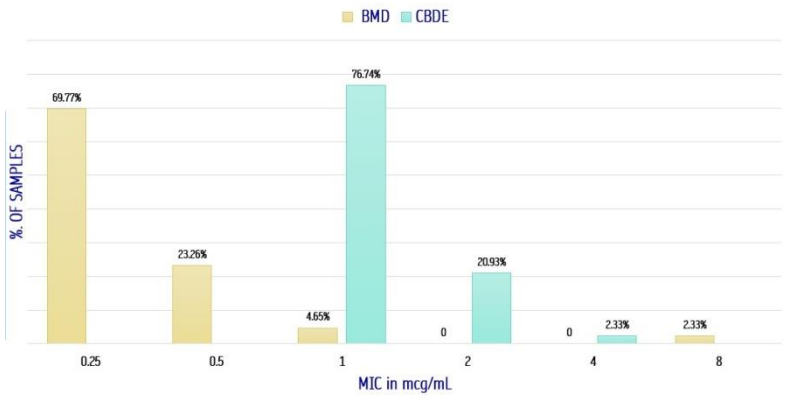

Fig.4 Graph comparing the percentage of isolates corresponding to specific MIC values determined by BMD and CBDE methods.

DISCUSSION

The present study aimed to evaluate and compare the efficacy of the Colistin Broth Disc Elution (CBDE) method with the Broth Microdilution (BMD) method for determining colistin susceptibility among carbapenemase-producing Enterobacteriaceae (CPE) isolates. Results from our study indicate a high level of agreement between the CBDE and BMD methods, highlighting the potential utility of CBDE as a

DOI: 10.69605/ijlbpr_14.3.2025.217

practical, cost-effective alternative to the more complex BMD method.

Our findings demonstrated a categorical agreement (CA) of 100% and an essential agreement (EA) of 97.67% between CBDE and BMD, which aligns closely with results obtained in other recent studies. Vijayapriya et al. reported similarly high concordance rates, with both CA and EA at 100% [1]. Their results strongly support the validity of CBDE as an accurate and reliable alternative to BMD, particularly valuable in resource-constrained settings.

Kar et al., investigating a modified version of the CBDE method (mCBDE), observed a slightly lower yet robust agreement, with a CA of 97.5% and an EA of 98.7% [2]. Although marginally lower than the present study, their findings further reinforce the reliability of disc elution methods in determining colistin susceptibility, especially when adaptations or modifications are utilized.

Földes et al. conducted a comparative study between CBDE and BMD, also reporting a CA of 100% [3]. Consistent with our results, their findings underscore the effectiveness of CBDE as an initial screening method. However, they caution that while CBDE is highly accurate for routine screening, it may have limitations in detecting specific resistance mechanisms, necessitating confirmation by BMD in cases with complex resistance profiles.

Contrasting our findings, Gupta et al. reported discrepancies in colistin susceptibility results when comparing non-BMD methods, such as E-test, Vitek 2, and disk diffusion, to the BMD method, with significant false susceptibility findings associated with these alternative methods [4]. In the present study, no such discrepancies were noted between CBDE and BMD. This strengthens the argument that CBDE offers greater accuracy and reliability compared to other non-BMD methods, thereby supporting its potential implementation in routine clinical microbiology laboratories for accurate colistin susceptibility testing.

CONCLUSION

The findings from the current study indicate that the Colistin Broth Disc Elution (CBDE) method is a promising, accurate, cost-effective, and reproducible alternative to the Broth Microdilution (BMD) method for determining colistin susceptibility in Carbapenemase-Producing Enterobacteriaceae (CPE). CBDE demonstrates a high degree of agreement with the reference BMD method, making it suitable for routine use, especially in resource-limited settings. However, it is recommended that CBDE be utilized primarily as a preliminary screening tool, with subsequent confirmatory testing using BMD or other established reference methods to ensure comprehensive accuracy and reliability in clinical decision-making.

LIMITATIONS

The study faces certain limitations, including a restricted sample size, which reduces the ability to generalize the findings across diverse clinical settings and populations.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this study.

REFERENCES

1. R. Vijayapriya, K. Lakshmi, M. Bhuvaneswari. A comparative study of colistin broth disk elution test and broth microdilution test on Gram-negative bacteria in all clinical isolates at tertiary care hospital, Tamil Nadu. Int J Acad Med Pharm. [Internet]. [cited 2024 Dec 26];1412–1415.
2. Kar P, Mahapatra A, Malik BA, Behera B,Mohanty S. Performance of modified colistin broth disc elution vis-à-vis broth microdilution method for susceptibility testing of Enterobacterales.J Lab Physicians. 2024;16:286-90. doi: 10.25259/JLP-2023-5-25 - (1801)
3. Földes A, Székely E, Voidazan 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;11(3):377. doi:10.3390/antibiotics11030377
4. Gupta P, Sharma R, Vyas A, Tak A. Comparative evaluation of broth microdilution with E-test, Vitek 2, and disk diffusion for susceptibility testing of colistin on Gram-negative bacteria. Indian J Med Sci. 2021;73(1):93.
5. Baskaran V, Jeyamani L, Thangaraju D, Sivaraj R, Jayarajan J. Detection of carbapenemase production by modified carbapenem inactivation method among carbapenem resistant gram-negative bacilli at a tertiary care centre in Coimbatore, Tamil Nadu, India. Natl J Lab Med. 2023 Jul;12(3):MO11-MO14. doi:10.7860/NJLM/2023/61092.2734.
6. World Health Organization. GLASS: the detection and reporting of colistin resistance. 2nd ed. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO
7. National Centre for Disease Control (NCDC), India. Standard Operating Procedure for Antimicrobial Resistance Surveillance: National AMR Surveillance Network (NARS-Net). 2nd ed. New Delhi: Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India; 2023.
8. Clinical and Laboratory Standards Institute. CLSI M100: Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2024. p. 192-195
9. Hindler JA, Schuetz AN, Abbott A, et al. A new phenotypic test for detection of carbapenemases – the modified carbapenem inactivation method (mCIM). CLSI AST News Update. 2017 Jun;2(1):7-8.
10. Joshi DN, Shenoy B, Bhavana MV, Adhikary R, Shamarao S, Mahalingam A. Prevalence of Carbapenem-Resistant Enterobacteriaceae and the Genes Responsible for Carbapenemase Production in a Tertiary Care Hospital in South India. EMJ. 2023 May.

DOI: 10.69605/ijlbpr_14.3.2025.217

11. HiMedia Laboratories. Colistin HiMIC™ Plate Kit MPK020: Antimicrobial Susceptibility Testing. Maharashtra, India: HiMedia Laboratories Pvt. Ltd.; 2024
12. Antimicrobial Agents Division, National and Regional Reference Laboratory for Antimicrobial Resistance, National Institute of Infectious Diseases – ANLIS "Dr. Carlos G. Malbrán". Colistin Micro Broth Disk Elution Test. Version EN 1.0. Argentina: ANLIS "Dr. Carlos G. Malbrán"; May 2020