Section: Microbiology

Original Research Article

COMPARATIVE EVALUATION OF COLISTIN BROTH DISK ELUTION TEST AND BROTH MICRO DILUTION TEST AMONG CARBAPENEMASE PRODUCING GRAM NEGATIVE BACILLI IN ALL CLINICAL ISOLATES OF A TERTIARY CARE HOSPITAL

L Siva Rajeswari[1], Sunitha B[2], KV Chakradhar[3]

[1]Post-Graduate, Department of Microbiology, NRI Institute of Medical Sciences, Sangivalasa, Visakhapatnam, Andhra Pradesh, India
[2]Assistant Professor, Department of Microbiology, NRI Institute of Medical Sciences, Sangivalasa, Visakhapatnam, Andhra Pradesh, India
[3]Professor, Department of Microbiology, NRI Institute of Medical Sciences, Sangivalasa, Visakhapatnam, Andhra Pradesh, India

Received : 11/08/2024
Received in revised form : 03/10/2024
Accepted : 17/10/2024

Keywords:
Micro dilution, carbapenemase, bacilli, tertiary care hospital.

Corresponding Author:
Dr. Sunitha B,
Email: dr.sunithavinod@gmail.com

DOI: 10.47009/jamp.2024.6.5.91

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2024; 6 (5); 482-486

Abstract

Background: Increased Antimicrobial resistance among clinically important Gram- negative bacilli, especially Carbapenemase-producing Enterobacterales and Pseudomonas, has regained interest in Polymyxin E (Colistin) and Polymyxin B as a last resort of treatment. Broth micro-dilution (BMD) is Colistin's only recommended susceptibility testing, but this method is impractical for most clinical laboratories. The study aims to evaluate the accuracy of the Colistin Broth Disk Elution test (CBDE) and the Broth Micro Dilution (BMD) method in obtaining Colistin MIC for Carbapenem-resistant Gram-negative bacilli (CRGNB) among all clinical isolates. **Materials and Methods:** A Cross-sectional descriptive study was conducted in the Department of Microbiology, NRIIMS, Vizag, from September 2022 to February 2023. During this period, 1496 clinical samples (including Blood, Purulent exudates, ET aspirates, and sterile body fluids) were collected by convenient sampling and processed. All Gram-negative bacilli isolates obtained from all age groups were included in our study. Repeated isolates from the same patient, isolates from stool samples and the organisms which are intrinsically resistant to Colistin, such as Proteus, Serratia, Providencia and Burkholderia species, were excluded from the study. Methodology: Carbapenem-resistant Gram-negative bacilli which were detected through the Kirby Bauer disk diffusion method using Ertapenem & Meropenem discs and CLSI breakpoints, were selected and then subjected to Colistin BMD according to the Standard operating procedure of the National Programme on AMR Containment, NCDC, India (August 2020) and CBDE test was conducted according to CLSI M100 S32 Performance standards. Results of CBDE were compared with BMD by using appropriate statistical tools. **Result:** Among 1496 clinical samples processed, 108 Carbapenem-resistant isolates were identified, with Klebsiella pneumoniae (31%) being the most common. Of which 35.1% are from exudates, followed by 20.3% from blood, 16.6% from ET aspirates,18.5% from Urine, 9.2% from BAL, and 5.5% from tissue samples. Most of the isolates (84) had an MIC ≤1, while 18 had an MIC of 2 and 4 had an MIC of >4 in both methods, with 2 isolates showing discordant results. The Colistin Broth Disk Elution test (CBDE) showed a high level of agreement with the Broth Micro Dilution (BMD) method, with a categorical agreement (CA) of 98% and an essential agreement (EA) of 100%. **Conclusion:** The study showed that the CBDE test can be used as an alternative to the BMD test for obtaining the MIC of Colistin, which is important for guiding the appropriate use of this last-resort antibiotic in clinical practice.

INTRODUCTION

Increased Antimicrobial resistance among clinically important gram-negative bacilli has been a major global threat since last two decades and especially emergence of carbapenemase producing Enterobacterales, Pseudomonas and Acinetobacter species has regained interest in polymyxins mainly polymyxin E(colistin) and polymyxin B as their last resort of treatment.[1-3]

But, unfortunately incidence of colistin resistance among them is also increasing now a days due to increased usage of this drug both in human and veterinary health care. As colistin is a large polycationic molecule which has poor diffusion capacity into agar thus, routinely performed disk diffusion or gradient diffusion tests cannot accurately detect colistin resistance.[4]

In 2015, both the Clinical and Laboratory Standard Institute (CLSI) and the European Committee of Antimicrobial Susceptibility Testing (EUCAST) joint working group has approved Broth Micro Dilution (BMD) as a reference method for antimicrobial susceptibility testing of colistin,[6] but it cannot be routinely performed as it is a labor intensive procedure and difficulty to perform due to polysorbate effectiveness and binding of colistin to microtitre plates, these properties made invitro susceptibility testing of colistin cumbersome and challenging. Since 2020 CLSI has also approved Colistin Broth Disk Elution Test (CBDE) and Colistin Agar Test (CAT) for colistin susceptibility testing (20).

There is a need of standardizing colistin testing method which is accurate and feasible for all clinical laboratories to perform. The present study is mainly conducted to evaluate accuracy of CBDE test with that of BMD test in obtaining Minimum Inhibitory Concentration (MIC) of colistin.

MATERIALS AND METHODS

A Cross sectional descriptive study was conducted in Microbiology Lab (Department of Microbiology NRIIMS, Visakhapatnam, South India). All Gram negative bacilli isolates which were obtained during the period of September 2022 to February 2023 from clinical samples of all age groups which were sent for routine diagnostic evaluation are included in our study and Repeated isolates from same patient, Isolates from stool samples, Organisms which are intrinsically resistant to colistin like Proteus, Serratia, Providencia and Burkholderia species were excluded from the study.

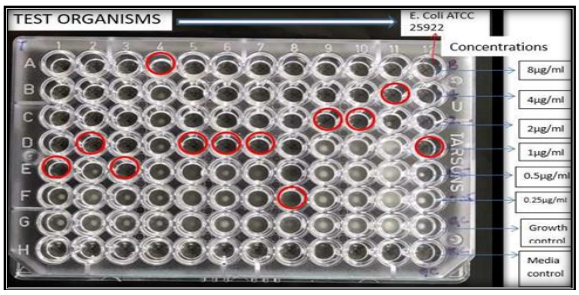
Methodology: During the study period 1496 clinical samples (including Blood, Purulent exudates, ET aspirates, Sterile body fluids, Urine) were collected and processed in our clinical laboratory. Out of them 452 Gram negative bacilli isolates were obtained.

Among them 108(23.8%) Carbapenem resistant isolates were detected through Kirby Bauer Disk Diffusion method using 10µg Meropenem discs and CLSI breakpoints. All the isolates were subjected to colistin BMD according to Standard Operating Procedure of Programme on AMR containment, NCDC, India (August 2020). For this procedure, Cation adjusted Muller Hinton Broth (CAMHB) media and colistin sulphate powder acquired from Himedia Laboratories, India. Stock solution (1,000 µg/ml) prepared by adding 10mg of colistin sulphate powder in 6.33ml of autoclaved distilled water. We have prepared 4 times of Working stock solution to attain the required concentration of colistin in microtiter wells. Desired concentration of Working solution (0.25 to 8µg/ml) were made by two fold serial dilutions.

For making 32µg/ml (4 X Working solution) from original stock solution of (1000µg/ml)
C1V1=C2V2
1000µg/ml X V1=32 µg/ml X 1000µl
V1=32µl

	500 µl →					
	1	1:2(2)	1:2(3)	1:2(4)	1:2(5)	1:2(6)
MCT	**968 sterile MHB +32µl working stock solution**	**500 sterile MHB**	**500 sterile MHB**	**500 sterile MHB**	**500 sterile MHB**	**500 sterile MHB**
Colistin Conc. (µg/ml) in MCT	32	16	8	4	2	1
From MCT take 25µl and add to all wells in corresponding microtiter plate column containing 50µl CAMHB						
Final conc. (µg/ml) in microtitre plate wells	8	4	2	1	0.5	0.25

Standardized inoculum of 0.5 McFarland was prepared using Direct colony suspension method. This inoculum is diluted 1:75 times by adding 10µL to 740µL of autoclaved MHB medium to yield bacterial concentration of 5x105 CFU/well. To achieve 100µL in each well of microtitre plate, test wells are added with 25µL of working solution, 25µL of inoculum and 50µL OF CAMHB ;75□L of CAMHB, 25□L of inoculum was added in Growth Control well and only 100□L CAMHB was added to Media Control well and incubated at 35 °C for 18 to 24hrs.

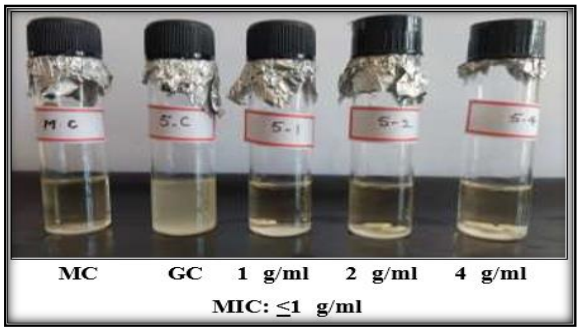


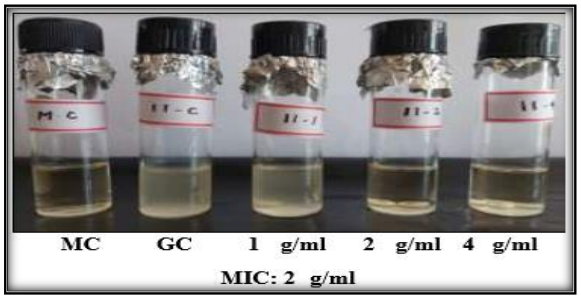
Colistin MIC's of test isolates in Broth Micro Dilution

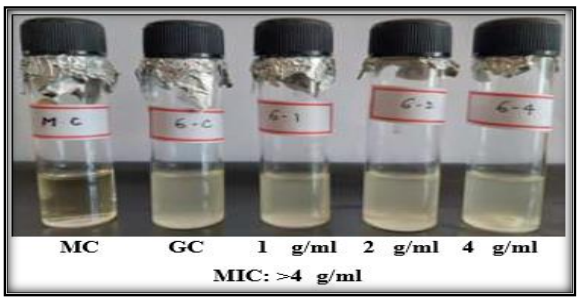
CBDE test was performed according to CLSI 2020 M100 S32 performance standards. For this procedure CAMHB and 10µg colistin sulfate discs were procured from Himedia Laboratories, India. 10ml of CAMHB was filled in each tube and 4 tubes are labeled as control, 1, 2, 4µg/ml. 1, 2 and 4 disks were added into tubes labelled 1, 2 and 4µg/ml

ISSN (O): 2687-5365; ISSN (P): 2753-6556

respectively. Then tubes are gently vortexed and had let the colistin elute from the disks for 30min to 1hour at room temperature. No disks were added to Growth control tube. 50µLof standardized inoculum of 0.5 McFarland added to each tube including Growth control tube and incubated at 35°C for 18 to 20hrs. For both methods, As Quality Control strains Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 and an In house Positive control strain were used.







Colistin Mic's of Test Isolates in CBDE Interpretation

The lowest concentration of drug at which the visible growth is completely inhibited after incubation is considered as MIC. Results of BMD and CBDE were interpreted based on colistin MIC breakpoints as recommended by 2021 CLSI guidelines. MIC results of CBDE were compared with that of reference BMD test. Agreement between CBDE and BMD were described as Categorical Agreement (CA) and Essential Agreement (EA).

RESULTS

Among 452 Gram negative bacilli isolates, 108(23.8%) carbapenem resistant isolates were identified with Klebsiella pneumoniae (34/108,31.4%) being most common then followed by Acinetobacter baumannii (28/108,25.9%), Escherichia coli (22/108,20.3%), Pseudomonas aeruginosa (20/108,18.5%), Klebisella oxytoca (4/108,3.7%) as shown in [Table 1]

Table 1: Distribution of Study isolates.

Organism	No. of isolates	Percentage
Klebsiella pneumoniae	34	31.4%
Acinetobacter baumannii	28	25.9%
Pseudomonas aeruginosa	20	18.5%
Klebsiella oxytoca	4	3.7%

Table 2: Sample wise distribution of CRGNB Isolate

Samples	Percentage of isolates obtained
Purulent exudates	35.1%
Blood	20.3%
E.T aspirates	16.6%
Urine	18.5%
BAL fluid samples	9.2%
Tissue samples	5.5%

Table 3: Distribution of Colistin MIC of CBDE with reference BMD

	MIC of BMD →						
MIC of CBDE ↓	**MIC(µg/ml)**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
	<1	8	30	46	0	0	0
	2	0	0	0	18	2	0
	4	0	0	0	0	2	
	>4	0	0	0	0	0	2

*Interpretation: <2 g/ml: Intermediate ;>4 g/ml: resistant.

Minor error(mE)-MIC: 4 g/ml (BMD)and2 g/ml (CBDE).

Table 4: Colistin MIC of CRGNB isolates in reference BMD

MIC	No. of isolates	Percentage
< 1µg/ml	84	77.7%
2 µg/ml	18	16.6%
>4 µg/ml	6	5.5%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 5: Organism wise distribution of colistin MIC

	MIC of BMD						
Organism	**No. of isolates tested**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
Klebsiella pneumoniae	34	0	4	16	10	2	2
Acinetobacter baumannii	28	2	12	8	4	2	0
Escherichia coli	22	4	4	12	2	0	0
Pseudomonas aeruginosa	20	0	8	10	2	0	0
Klebsiella oxytoca	4	2	2	0	0	0	0

As shown in [Table 2], Majority of isolates were obtained from Purulent exudates (35.1%), Blood (22/108,20.3%), ET aspirate (18/108, 16.6%), Urine (18.5%), BAL fluid (10/108,9.2%), Tissue samples (6/108,5.5%).

Majority of the isolates were obtained from patient suffering with Surgical Site Infections (32/108,29.6%), Ventilator Associated Pneumonia (18/108,16.6%), Sepsis (11.1%), Pulmonary disease (10.4%), Chronic kidney disease (8.2%), Poly trauma (5.5%), others (18.5%).

Out of 108, 84 isolates (77.8%) had MIC <1µg/ml, while 18 isolates (16.7%) had MIC of 2µg/ml and 4 isolates (3.7%) had MIC >4µg/ml in both methods, with 2 isolates (1.8%) showed MIC of 2 in test method and 4 in reference BMD. Out of 108 CRGNB, 6(5.5%) isolates showed resistance for colistin by BMD. Results in our study represents CBDE test has an EA of 100% and CA of 98.1%.

DISCUSSION

Carbapenem resistant Gram-negative bacilli has gained major importance in health care system as they are mainly associated with nosocomial infections and are difficult to treat. Polymyxins are considered as last choice of antibiotics for CRGNB infections. Especially colistin which is widely used in both human and veterinary health which lead to development of colistin resistance. And due to lack of a feasible test for colistin susceptibility testing, most of the laboratories cannot perform appropriate susceptibility testing for colistin.

This study is mainly conducted to assess the accuracy of CBDE test with that of reference colistin BMD. Prevalence of Carbapenem resistant GNB in the present study was 23.8%, which is correlated with Nair and vaz et al,[12] who reported 26%,

K. Sreeja vamsi et al,[13] reported 38.3% in their respective studies.

In the present study, majority of isolates with carbapenem resistance was observed in patients of Surgical ICU and wards (35.1%), which is correlated with Namitha Thomas et al,[14] reported 36.2%, Dr. Elandevi et al,[15] reported 47.1%, Uddin mohammad et al,[16] reported 23%.

In the present study, Prevalence of colistin resistance among carbapenem resistant GNB in our study was 5.5%, which is correlated with Ayushi sharma et al,[17] reported 6.2%, Sujatha et al,[3] and Kar et al,[19] reported 11% and 14% of colistin resistance respectively.

In the present study, CA of CBDE test was 98% and EA was 100% with respect to reference BMD, which is correlated with Sujatha et al,[3] reported 98%, Swati sharma et al,[18] reported 98.4%, Simner et al,[7] reported 100% in their studies.

CONCLUSION

We here by conclude that our study showed a high degree of agreement between the two methods.

Therefore CBDE test can be used as an alternative to BMD test in obtaining MIC for colistin, which is important for guiding appropriate use of last resort antibiotic in clinical practice.

REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100 (ISBN 978-1-68440-134-5; ISBN 978-1-68440-135-2. Clinical and Laboratory Standards Institute, USA, 2022.
2. Falagas ME, Kasiakou SK. Colistin: The revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin. Infect. Dis. 2005;40:1333-1341.
3. Sujatha SR, Deepashree R, Tejashree A, Sai S. Evaluation of Colistin Broth Disk Elution and Colistin Agar test: A study from teritary care hospital, South India. J Pure Appl Microbiol. 2022;16(2):885-890.
4. Stalin MJ. The search for practical method for colistin susceptibility testing: Have we found it by going back to future? J Clin Microbiol. 2019;57(2):e01608-18.
5. Humphries RM, Green DA, Schuetz AN, et al. Multicentre evaluation of colistin broth disk elution and colistin agar test: a report from the Clinical and Laboratory Standards Institute. J Clin Microbiol. 2019;57(11):e01269-19.
6. Recommendations for MIC determination of colistin (Polymyxin E) as recommended by joint CLSI-EUCAST Polymyxin breakpoints Working Group.
7. Simner PJ, Bergman Y, Trejo M, et al. Two site evaluation of colistin broth disk elution test to determine in vitro activity against Gram negative bacilli. J Clin Microbiol. 2019;57(2):e01163-18.
8. Sarumathi D, Rajashekar D, Sastry AS. Comparision of antimicrobial Susceptibility Testing Methods for Colistin against Carbapenem Resistant Enterobacteriacae in a Teritary Care Hospital of Southern India. J Clin Diagn Res. 2020;14(7):DC01-DC05.
9. Broth-microdilution Colistin Susceptibility Test for Aerobic Gram Negative Bacteria-Standard Operating Procedure; National Programme on Antimicrobial Resistance Containment National Centre for Disease Control, India, 2020. http://ncdc.gov.in/Write Read Data/1892s/82259485491600061012.pdf.
10. Dalmolin TV, Mazzetti A, Ávila H, Kranich J, Carneiro GIB, Arend LNVS, et al. Elution methods to evaluate colistin susceptibility of Gram-negative rods. Diagn Microbiol Infect Dis. 2020;96:114-910.
11. Bassetti M, Peghin M, Vena A, Giacobbe DR. Treatment of Infections Due to MDR Gram-Negative Bacteria. Front. Med. (Lausanne). 2019;6:74. Doi: 10.3389/fmed.2019.00074.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

12. Nair PK, Vaz MS. Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai. India J of Microbiology and Infectious Diseases. 2013;3(4):207-210.
13. Sreeja Vamsi K, Ramamoorthy S, Murali TS, Abhishek Vamsi B. Ramachandra Reddy and Mary Hameliamma. Prevalence of Carbapenem Resistant Gram- Negative Bacteria in Rural Hospital Mahabubnagar, Telangana and Systemic Review. Int. J Curr. Microbiol. App. Sci. 2021;10(03):1542-1547. Doi: https://doi.org/10.20546/ijcmas.2021.1003.190.
14. Namitha Thomas, Tarana Sarwat. Prevalence of Carbapenem Resistant Enterobacteriaceae in A Tertiary Care Hospital. Int. J Curr. Microbiol. App. Sci. 2019;8(11):1418-1424. Doi: https://doi.org/10.20546/ijcmas.2019.811.166
15. Dr. Elandevi Ramalingam, Dr. Kalyani Mohanram, Dr. Himadri Dutta. A Study on Phenotypic Characterization of Carbapenemases among Gram Negative Bacilli in a Teritary care hospital. JMSCR. 2016 Nov;4(11):14057-14065.
16. Mohammad MU, Manisha DR, Nagamani K. Clinical, Phenotypic and genotypic profile of carbapenem resistant Gram-negative infections in intensive care units. Indian J Microbiol Res. 2021;8(1):28-34.
17. Sharma A, Agarwal M. Colistin susceptibility for carbapenem resistant Gram negative Baclli; Comparative study of E-test and Vitek 2 compact with Broth microdilution. Galore International Journal of Health Sciences and Research. 2019;4(4):110-115.
18. Swati Sharma, Tuhina Banerjee, Rahul Garg, Padma Das. Evaluation Report of the Colistin Broth Disk Elution Method with Acinetobacter baumannii isolates from a Low Resource Setting. Microbiology Spectrum, 2022. 10;10.1128/spectrum.00871-22.
19. Punyatoya Kar, Bijayini Behera, Srujanu Mohanty, Jayanti Jena, Ashoka Mahapatra. Detection of colistin resistance in Carbapenem Resistant Enterobacteriaceae by Reference Broth microdilution and Comparative evaluation of Three other methods. Journal of Laboratory Physicians. 2021;13:263-269.
20. Wayne PA. Clinical and Laboratory Standards Institute Performance standards for Antimicrobial Susceptibility Testing 31st edition, 2021.
21. Walia K, Madhumathi J, Veeraraghavan B, et al. Establishing antimicrobial resistance surveillance and research network in India: Journey so far. Indian J Med Res. 2019;149(2):164-179.

ISSN (O): 2687-5365; ISSN (P): 2753-6556