Section: Microbiology

Original Research Article

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, TAMILNADU

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Received : 17/11/2023
Received in revised form : 14/01/2024
Accepted : 31/01/2024

Keywords:
Gram-negative bacterial infections, Colistin broth disk elution test, Broth microdilution test, Colistin, Multidrug resistance.

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DOI: 10.47009/jamp.2024.6.1.281

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

Int J Acad Med Pharm
2024; 6 (1); 1412-1415

Abstract

Background: Gram-negative bacterial infections are a significant public health challenge due to high morbidity and antibiotic resistance. Colistin, a polymyxin derivative, has lost usage due to nephrotoxicity and neurotoxicity. Aim: This study was done to evaluate the accuracy of the Colistin Broth Disk Elution test (CBDE), which is user-friendly with that of the more cumbersome Broth Micro Dilution (BMD) test in obtaining Colistin MIC for all clinical Gram-negative bacterial isolates. **Material and Methods:** This comparative study was conducted on gram-negative bacterial isolates from October to December 2023 in the Microbiological Department of tertiary care Government Hospital of Trichy. Cation-adjusted Mueller-Hinton broth was used to measure the BMD and CBDE. The BMD and CBDE results were interpreted based on colistin MIC breakpoints, as the 2021 CLSI guidelines recommended. **Results:** Escherichia coli was the most commonly isolated organism in nearly half of the samples tested. Klebsiella pneumoniae was next with nearly one-fifth, followed by acinetobacter contributing 17.3%, and the least among identified was pseudomonas at 13.4%. Among the 276 samples, 197 had a MIC value of 0.25 (71.4%), and 63 had a MIC value of 0.5 (22.8%). The results of both methods showed that an MIC value of less than or equal to one was nearly similar in both tests (71.4%, 22.8%, 5.4%, and 98.9%). **Conclusion:** CBDE is recommended by the CLSI 2021 and is inexpensive. The CBDE method was reproducible and accurate. It 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 resource-limited settings.

INTRODUCTION

Gram-negative bacterial infection is clinically important in hospitals and also a significant public health challenge due to its high morbidity among those infected and high resistance to antibiotics, requiring patients to be in the intensive care unit. It also has a high risk of mortality.1 While colistin, a polymyxin derivative, is among the first antibiotics that significantly act on gram-negative bacteria and has been on the market for more than fifty years, it had lost its usage due to concerns over its nephrotoxicity and neurotoxicity, getting replaced by aminoglycosides in the 70s.2 The silent tsunami facing modern medicine is antibiotic resistance, especially the rise of multidrug resistance among gram-negative bacteria, a serious challenge among healthcare professionals.3 Colistin is used as last-line therapy for multidrug-resistant gram-negative bacteria with no alternative antibiotics.2,10

Recently, the resistance to colistin among clinical isolates has been frequently reported due to its increased usage, making it essential to carry out susceptibility testing for Colistin among Gram-negative bacteria-positive samples for surveillance and treatment.4 The CLSI-EUCAST has recently recommended using sulphate salt of colistin and standard polystyrene trays without surfactants for testing as per ISO standards.5 The Clinical and Laboratory Standard Institute 2016 recommended broth dilution as a reference standard for Colistin

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

susceptibility testing.6 The reference broth dilution tests are rarely conducted in clinical laboratories due to its requirement to have freshly prepared or frozen antibiotic solutions, which is a laborious process.7

Aim

This study was done to evaluate the accuracy of the Colistin Broth Disk Elution test (CBDE), which is user-friendly with that of the more cumbersome Broth Micro Dilution (BMD) test in obtaining Colistin MIC for all clinical Gram-negative bacterial isolates.

MATERIALS AND METHODS

This comparative study was conducted on gram-negative bacterial isolates from October to December 2023 in the Microbiological Department of tertiary care Government Hospital of Trichy, Tamil Nadu.

Inclusion Criteria

All Gram-negative bacterial isolates obtained from October to December 2023 were included in this study.

Exclusion Criteria

Repeated isolates from the same patient, stool samples, and organisms intrinsically resistant to colistin, such as Proteus, Serratia, Providencia, and Burkholderia species, were excluded from the study.

All gram-negative bacterial isolates were subjected to broth microdilution (BMD) and colistin broth disk elution (CBDE). Cation-adjusted Mueller-Hinton broth was used to measure the BMD and CBDE. The results of the CBDE were compared with those of the BMD by applying the required statistical tools. According to the standard operating protocol issued by the National Programme on Antimicrobial Resistance Containment National Centre for Disease Control, India, reference in-house BMD was performed on a polystyrene microtitre plate. The CBDE test was performed according to CLSI 2020 M100 S32 performance standards. The test was performed using the appropriate control strains. For quality control, E. coli ATCC 25922 was used as recommended by CLSI 2021.

This test involved using small volumes of broth dispensed in sterile microdilution plates with conical bottom wells. Each well contained 0.1 ml of broth.

1. 0.1 (± 0.02) of broth containing antibiotics was added to each well. A growth and sterility control well (uninoculated wells) was included.
2. The plates were sealed in plastic bags and frozen at ≤ -20°C.
3. On the day of testing, the panels were inoculated at a standard density of 5 × 105 CFU/ml.
4. The plate was sealed in a plastic bag before intubation to prevent drying.
5. It was then incubated for 16-20 hours at 35 ± 2°C before visual determination of MICs.

Colistin Disk Elution Test

This method used four glass tubes, and 10 mL of Cation-adjusted Muller Hinton broth (HI-media) was added to each tube. The first tube was used as a growth control (no antibiotic disc was added). One disc of colistin sulphate (10 µg) (Oxoid) was added to the second tube. Two discs of colistin sulphate (10 µg) were added to the third tube, and four discs of colistin sulphate (10 µg) were added to the fourth tube. The tubes were incubated at room temperature for 30-45 minutes to elute colistin from the medium. Colonies from blood agar were used to prepare a 0.5 McFarland solution in normal saline, and after mixing properly, 50 µL inoculum was added to each tube. The test tubes were mixed thoroughly and incubated at 37 °C for 24 h. The colistin MIC results were interpreted as per CLSI-202.

Interpretation

The Minimum Inhibitory Concentration (MIC) breakpoints of colistin, as per the recommendations of CLSI 2021, were used in the interpretation. Here, MIC was the lowest drug concentration at which visible growth was inhibited after incubation. The MIC results of CBDE were compared with those of the gold-standard BMD test.

Data Analysis

Data entry was performed using WHONET 2023, and analysis using SPSS software version 29.0. The BMD and CBDE results were interpreted based on colistin MIC breakpoints, as the 2021 CLSI guidelines recommended.

RESULTS

A total of 451-gram negative bacilli isolates were obtained from 7452 samples collected during the study period. Based on the exclusion criteria, 276 eligible samples were included in the study, with the highest number of urine samples, as shown in Table 1, followed by pus. The sputum samples also included endotracheal aspirates and bronchial wash, accounting for just over 11%, whereas body fluids and blood samples accounted for 7% each. [Table 1]

Table 2 shows the distribution of organisms identified among the study isolates, where Escherichia coli was the most commonly isolated organism in nearly half of the total samples tested. Klebsiella pneumoniae was next with nearly one-fifth, followed by acinetobacter contributing 17.3%, and the least among identified was pseudomonas at 13.4%.

Among the 276 samples, 197 had a MIC value of 0.25 (71.4%), 63 had a MIC value of 0.5 (22.8%), and 15 had a MIC value of 1 (5.4%), and one had a MIC of 8 (0.4%). [Table 2]

Tables 3 and 4 show the results of tests performed using both Colistin Broth Microdilution and Colistin Broth Elution Disk methods with their MIC values. [Table 3]

The results of both methods showed that an MIC value of less than or equal to one was nearly similar

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

in both tests (71.4%, 22.8%, 5.4%, and 98.9%). [Table 4]

Table 1: Distribution of gram-negative isolates included in the study based on the sample type

Type of sample	No of isolates	Percentage
Urine	121	43.8
Pus	89	32.2
Sputum (including ET aspirate and bronchial wash)	32	11.6
Body fluids	20	7.2
Blood samples	19	6.9

Table 2: Distribution of study isolates based on organism

Organism	Number of isolates	Percentage
Escherichia coli	136	49.3
Klebsiella pneumoniae	55	19.9
Acinetobacter baumannii	48	17.3
Pseudomonas aeruginosa	37	13.4

Table 3: Distribution of study isolates based on MIC by Colistin Broth Microdilution

Organism	Number of Isolates	MIC Value					
		0.25	0.5	1	2	4	8
Escherichia coli	136	128 (46.4%)	7 (2.5%)	1 (0.3%)	0	0	0
Klebsiella pneumoniae	55	27 (9.8%)	20 (7.2%)	7 (2.5%)	0	0	1 (0.3%)
Pseudomonas aeruginosa	37	30 (10.9%)	5 (1.8%)	2 (0.7%)	0	0	0
Acinetobacter baumannii	48	12 (4.3%)	31 (11.2%)	5 (1.8%)	0	0	0
Total	276	197 (71.4%)	63 (22.8%)	15 (5.4%)	0	0	1 (0.3%)

Table 4: Distribution of study isolates based on MIC by Colistin Broth Elution Disk

Organism	Number of Isolates	MIC Value		
		≤1	2	≥4
Escherichia coli	136	136 (49.3%)	0	0
Klebsiella pneumoniae	55	54 (19.6%)	0	1 (0.3%)
Pseudomonas aeruginosa	37	36 (13%)	0	1 (0.3%)
Acinetobacter baumannii	48	47 (17%)	0	1 (0.3%)
Total	276	273 (98.9%)	0	3 (1.1%)

DISCUSSION

CBDE applies the same principle that was used to determine anaerobe antimicrobial susceptibility in 1973, where antimicrobial disks of a known concentration were eluted in a set volume of broth, to obtain standard doubling dilutions to determine MICs (18). CLSI and EUCAST currently recommend that colistin AST be performed by rBMD without surfactant, a method few laboratories have access to.[8,9]

Although rBMD is an accurate method for colistin MIC determination, it can be resource intensive for clinical microbiology laboratories. Disk and gradient diffusion methods are not recommended by either CLSI or EUCAST for testing colistin due to unacceptably high error rates,[10,11] leaving microbiology laboratories without a practical method to identify colistin susceptibility.

In this study, there was 100% concordance in the detection of Colistin susceptibility test belonging to Enterobacteriaceae by both the phenotypic method similar to the study by Chauhan et al.[12]

Also, one isolate from Acinetobacter baumannii and pseudomonas that showed as susceptible to BMD became Resistant to CBDE, which is also similar to the study by Chauhan et al.[12]

The Colistin Broth Disk Elution test (CBDE) showed a high level of agreement with the Broth Micro Dilution (BMD) method, with a definite agreement (CA) of 98% and an essential agreement (EA) of 100%, which is in concordance with the study performed by Rajeswari et al.[13]

CONCLUSION

Gram-negative bacterial infections are associated with a higher rate of morbidity and mortality in ICUs. The most challenging are those with multidrug resistance, for which colistin is the last line of available drugs. A feasible method for the routine testing of colistin among gram-negative clinical isolates is essential for both treatment and surveillance.

With the challenges hindering the routine use of the gold standard test of the Broth Microdilution (BMD) method, a more user-friendly yet sensitive test for colistin resistance in all laboratories is necessary. CBDE is recommended by the CLSI 2021 and is inexpensive. The CBDE method was reproducible and accurate. It 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 resource-limited settings.

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