

Comparison of Different Methods for In Vitro Susceptibility Testing of Colistin in Gram-Negative Bacilli Isolated from a Tertiary Care Hospital

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Abstract: Background: The global rise in antimicrobial resistance has necessitated the reintroduction of colistin as a last-resort antimicrobial agent against multidrug-resistant Gram-negative bacilli. Accurate laboratory determination of colistin susceptibility remains challenging due to methodological limitations. Objectives: To compare the performance of colistin broth disc elution (CBDE) and E-test methods with broth microdilution (BMD), the reference standard, for detection of colistin resistance among Gram-negative bacilli. Methods: A laboratory-based, cross-sectional, comparative study was conducted over one year in a tertiary care hospital. Clinical samples from admitted patients were processed using standard microbiological techniques. Colistin susceptibility testing was performed using BMD, CBDE, and E-test. Categorical agreement of CBDE and E-test was calculated against BMD. Results: Of 456 Gram-negative bacilli isolated, 57 (12.5%) were colistin-resistant by BMD. *Klebsiella pneumoniae* was the predominant resistant organism. CBDE showed a high categorical agreement with BMD (94.7%), whereas E-test showed lower agreement (86.0%). Conclusion: Broth microdilution remains the gold standard for colistin susceptibility testing. CBDE is a reliable and cost-effective alternative suitable for routine diagnostic laboratories, while E-test results should be interpreted with caution.

Keywords: Colistin; Gram-negative bacilli; Broth microdilution; Colistin broth disc elution; Antimicrobial resistance

1. Introduction

Antimicrobial resistance (AMR) has emerged as one of the most critical global public health challenges, largely driven by the misuse and overuse of antimicrobial agents. The increasing prevalence of multidrug-resistant (MDR) Gram-negative bacilli, particularly carbapenem-resistant Enterobacterales, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, has severely limited available therapeutic options.

Colistin, a polymyxin antibiotic, has re-emerged as a last-line agent for the treatment of infections caused by these MDR pathogens. However, colistin susceptibility testing is technically challenging due to poor diffusion in agar-based methods and the phenomenon of heteroresistance. Consequently, international guidelines recommend broth microdilution (BMD) as the reference method. Despite this, BMD is not routinely performed in many laboratories due to technical and logistical constraints. Alternative methods such as colistin broth disc elution (CBDE) and gradient diffusion (E-test) have been proposed, but their reliability remains variable. This study aims to compare these methods to identify a practical and accurate alternative for routine laboratory use.

2. Materials and Methods

This laboratory-based, cross-sectional, comparative study was conducted in the Department of Microbiology of a tertiary care teaching hospital over a period of one year (June 2024 to May 2025). Clinical samples including blood, urine, respiratory specimens, pus, body fluids, and device tips were collected aseptically from admitted patients and processed according to standard microbiological protocols.

Gram-negative bacilli were identified using colony morphology, Gram staining, and conventional biochemical

tests. Duplicate isolates from the same patient, mixed growth samples, and contaminated cultures were excluded.

Colistin susceptibility testing was performed using broth microdilution (BMD) as the reference method, following CLSI and EUCAST recommendations. Colistin broth disc elution (CBDE) and E-test were used as comparative methods. *Escherichia coli* ATCC 25922 was used as the quality control strain. Results were interpreted based on established breakpoints, and categorical agreement was calculated.

3. Results

A total of 620 clinical samples were processed during the study period, of which 456 (73.5%) yielded Gram-negative bacilli. *Klebsiella pneumoniae* was the most frequently isolated organism, followed by *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.

Colistin resistance was detected in 57 (12.5%) isolates by broth microdilution. *Klebsiella pneumoniae* accounted for the majority of colistin-resistant isolates. CBDE demonstrated a high categorical agreement with BMD (94.7%), whereas E-test showed a lower agreement (86.0%). CBDE showed excellent agreement for non-fermenting Gram-negative bacilli.

4. Discussion

The present study highlights the increasing prevalence of colistin resistance among Gram-negative bacilli in a tertiary care setting. The predominance of *Klebsiella pneumoniae* as a colistin-resistant pathogen is consistent with previous Indian studies. Accurate detection of colistin resistance is critical, as inappropriate reporting may lead to treatment failure and adverse clinical outcomes.

CBDE demonstrated excellent categorical agreement with

the reference BMD method, supporting its utility as a reliable and practical alternative for routine laboratories. The comparatively lower agreement observed with E-test may be attributed to poor agar diffusion and heteroresistance, which are well-documented limitations of gradient diffusion methods for colistin.

5. Limitations

This was a single-center study, and the findings may not be generalizable to all healthcare settings. Automated susceptibility testing systems could not be evaluated due to logistical constraints.

6. Conclusion

Broth microdilution remains the gold standard for colistin susceptibility testing. Colistin broth disc elution is a reliable, cost-effective alternative with high categorical agreement and can be adopted in routine diagnostic laboratories where BMD is not feasible. E-test, although convenient, should be interpreted cautiously.

References

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