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Modified Colistin Broth Disc Elution: A Novel Method To Identify Colistin Susceptibility In Enterobacterales

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ABSTRACT

Background: Colistin is a critical last-resort antibiotic for multidrug-resistant (MDR) Gram-negative pathogens, particularly Enterobacterales. Accurate susceptibility testing is essential but limited by the labor-intensive, resource-demanding broth microdilution (BMD) method and unreliable disk diffusion or automated assays.

Aim: To evaluate a microplate-based modified Colistin Broth Disc Elution (mCBDE) method as a cost-effective, high-throughput alternative to BMD for colistin susceptibility testing in clinical MDR Enterobacterales isolates.

Methods: One hundred twenty non-duplicate MDR Enterobacterales isolates from various clinical specimens underwent parallel susceptibility testing by reference BMD (CLSI M100) and mCBDE in 96-well microplates. For mCBDE, 10 µg colistin discs were eluted in cation-adjusted Mueller–Hinton broth to produce concentrations of 0, 1, 2, and 4 µg/mL. Standardized inocula (~7.5 × 10^5 CFU/mL) were incubated at 35 ± 2 °C for 16–20 hours. MICs were interpreted per CLSI breakpoints (≤2 µg/mL susceptible; >2 µg/mL resistant). Categorical agreement (CA), very major error (VME), and major error (ME) rates were calculated. Agreement was assessed by Cohen's kappa (κ) with 95% confidence interval.

Results: mCBDE achieved 90.8% CA with BMD. Among 14 BMD-resistant isolates, mCBDE correctly identified 11 and missed 3 (VME = 2.9%). Of 106 BMD-susceptible isolates, mCBDE correctly classified 99 and produced 7 false resistances (ME = 6.6%). MIC distributions were comparable: BMD identified 81.6% of isolates with MIC ≤1 µg/mL versus 80.8% by mCBDE; 6.7% versus 4.2% at 2 µg/mL; 7.5% at 4 µg/mL by both methods; and 4.2% versus 7.5% above 4 µg/mL. Cohen's κ of 0.64 (95% CI: 0.50–0.78) indicated substantial agreement. The microplate format reduced reagent volume by over 75% and hands-on time by 50% versus BMD.

Conclusion: The microplate-based mCBDE method is a reliable, efficient, and economical alternative to BMD for colistin susceptibility testing in MDR Enterobacterales. Its streamlined workflow and resource savings support routine adoption, particularly in resource-limited settings, with confirmatory BMD recommended for isolates at clinical breakpoints.

Keywords: *Colistin; Enterobacterales; Drug Resistance, Multiple; Microbial Sensitivity Tests; Broth Dilution, Microtiter; Cohen's Kappa.*

INTRODUCTION

Carbapenem-resistant Gram-negative bacteria pose a global issue, with polymyxins being the most depended upon treatment approach. In 2017, the WHO classified colistin as a medication in the "RESERVE" category, to be utilized solely in the most critical situations when all other options have been exhausted.[1] These bacteria are implicated in various human illnesses, occurring in both community and hospital environments, and often exhibit co-resistance to multiple classes of antibiotics essential for treatment.[2] Colistin susceptibility should be assessed in routine antibiogram panels in environments where multidrug-resistant Gram-negative bacilli are common to prevent reporting delays. Rapid and reliable colistin susceptibility testing procedures are essential for effective therapeutic decision-making in routine diagnostic laboratories.[3]

Colistin is an effective drug against aerobic Gram-negative pathogens that often constitute the primary cause of life-threatening infections, including carbapenem-resistant *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli*, and other *Enterobacteriaceae.* It is significant that certain bacterial species, including *Serratia marcescens, Proteus* spp., *Providencia* spp., *Morganella morganii, Vibrio cholerae, Brucella, Campylobacter*, *Legionella, Chromobacterium*, *Neisseria* spp., *Edwardsiella* spp., various *Aeromonas* species, *Burkholderia cepacia*, anaerobic Gram-negative cocci, eukaryotic microbes, and mammalian cells, exhibit intrinsic resistance to colistin.[4] Colistin primarily exerts its bactericidal effects by compromising the integrity of the cell membrane in Gram-negative bacteria. Through electrostatic interactions and cationic displacement of calcium and magnesium ions from lipopolysaccharides, colistin destabilizes the membrane, enhances its permeability, induces leakage of cellular contents, and activates cell death pathways. Colistin may exert additional bactericidal effects through (i) the neutralization of lipopolysaccharides (LPS), the endotoxin of Gram-negative bacteria, and/or (ii) the suppression of bacterial respiration.[5]

Resistance to colistin can be classified into two categories: mutational resistance and transferable resistance. The mechanisms underlying mutational resistance encompass diminished binding to positively charged lipopolysaccharides (LPS) resulting from the incorporation of cationic groups (phosphoethanolamine (pEtN), 4-amino-4-deoxy-L-arabinose (L-Ara4N)), chromosomal modulation of the PmrAB and PhoPQ regulatory systems, loss of the binding site due to mutations in the LPS synthesis gene, reduced binding to the target site due to dysregulation of capsular polysaccharide synthesis, and the activity of efflux pumps. Transferable resistance, conversely, arises via plasmid-mediated mcr (mobile colistin resistance) genes.[6]

Given this context, accurate colistin susceptibility testing is crucial for guiding effective therapy and controlling the spread of resistance. Unfortunately, laboratory detection of colistin resistance faces technical hurdles. Colistin susceptibility testing by disk-diffusion method is unreliable due to poor diffusion of colistin into agar, yielding smaller zones of inhibition. The Clinical Laboratory Standard Institute (CLSI) recommends colistin agar test, colistin broth disk elution (CBDE) and colistin broth microdilution (BMD) MICs methods for in-vitro susceptibility testing of colistin in *Enterobacterales* and *Pseudomonas aeruginosa*.[7] However, this method demands expert handling, and is not feasible for many laboratories, especially in resource-limited settings.

To address these operational challenges, alternative phenotypic protocols have been developed. The modified CBDE method (mCBDE) utilized in the present study adapts the traditional broth disc elution protocol to a microtiter plate format, allowing simultaneous testing of multiple isolates with reduced reagent use and volume. Colistin discs are eluted in cation-adjusted Mueller–Hinton broth, and aliquots are added to microplate wells, streamlining preparation and increasing throughput. This modification improves efficiency and practicality in routine laboratories while maintaining comparable performance to reference BMD for colistin susceptibility testing.

The present study addresses the challenge of accurate colistin susceptibility testing, essential for managing multidrug-resistant Gram-negative infections. Traditional methods like disk diffusion and automated systems are unreliable, while the gold standard BMD is labor-intensive and impractical for routine use. This study evaluates the mCBDE method in a microplate format as a simpler, cost-effective alternative. The primary aim was to assess mCBDE's diagnostic accuracy, sensitivity, specificity, and agreement with BMD in clinical multidrug-resistant Enterobacterales isolates, seeking a reliable and accessible option to enhance colistin resistance detection and clinical decision-making.

MATERIALS AND METHODS

Study Design and Setting

This prospective, laboratory-based, cross-sectional diagnostic accuracy study was conducted in the Department of Microbiology, PSG Institute of Medical Sciences & Research (PSG IMS&R), following institutional ethics committee approval. The investigation aimed to evaluate the diagnostic performance of a modified colistin broth disc elution (mCBDE) protocol using a microtiter plate format for colistin susceptibility testing in clinical isolates of multidrug-resistant (MDR) Enterobacterales.

Study Population and Sample Size
A total of 120 non-duplicate MDR Enterobacterales isolates were collected from routine clinical specimens received in the Department of Microbiology during the study period. The sample size calculation was based on prevalence estimates from institutional data. All isolates underwent simultaneous testing using both the reference BMD method and the modified CBDE protocol.

Inclusion and Exclusion Criteria
Carbapenem-resistant *Enterobacterales* pathogens isolated from all clinical specimen types, including blood cultures, urine, endotracheal/tracheostomy aspirates, pus, sterile body fluids, and wound specimens were included in the present study. Pathogens demonstrating intrinsic resistance to colistin (such as *Proteus* species, *Morganella* species, *Providencia* species, and *Serratia marcescens*) and carbapenem-sensitive isolates were excluded from the analysis.

Modified Colistin Broth Disc Elution Protocol
The mCBDE methodology represented a microplate-based adaptation of the conventional CBDE technique. Four separate tubes, each containing 10 mL of single-strength cation-adjusted Mueller-Hinton broth (CA-MHB) (HiMedia, Mumbai, India), were prepared. Colistin discs (10 µg each, HiMedia, Mumbai) were added to achieve theoretical final concentrations: 0 discs (growth control), 1 disc (1 µg/mL), 2 discs (2 µg/mL), and 4 discs (4 µg/mL). The tubes were incubated at room temperature for 30 minutes to ensure adequate antibiotic elution from the discs.

Following elution, microtiter plates were labeled according to drug concentrations, and 200 µL aliquots of each prepared concentration were dispensed into designated wells. Standardized bacterial inocula (0.5 McFarland standard) were prepared from 18-24 hour cultures, and 3 µL volumes were added to each well to achieve a final bacterial concentration of approximately 7.5×10^5 CFU/mL. Plates were incubated overnight at 37°C under ambient atmospheric conditions.

Quality Control
Standard reference strains were incorporated for quality assurance: Escherichia coli NCTC 13846 (mcr-1 positive, colistin-resistant control) and E. coli ATCC 25922 (colistin-susceptible control) were tested concurrently with each batch of clinical isolates.

Interpretation Criteria
MICs were determined as the lowest concentration demonstrating complete absence of visible bacterial turbidity after 16-20 hours of incubation. Results were interpreted according to current Clinical and Laboratory Standards Institute (CLSI) guidelines: isolates with MICs ≤2 µg/mL were classified as intermediate (susceptible), while those with MICs >2 µg/mL were considered resistant. However, it should be noted that CLSI has recently revised colistin breakpoints, establishing an intermediate-only category (≤2 µg/mL) with no susceptible category due to clinical and pharmacokinetic limitations.

Reference Broth Microdilution Method
The gold standard BMD protocol was performed according to established CLSI M100 guidelines. Serial dilutions of colistin sulfate were prepared in CA-MHB using standardized techniques, and MICs were determined following overnight incubation at 35 ± 2°C.

Statistical Analysis
Statistical analysis was performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies, while continuous variables were expressed as mean ± standard deviation or median (range) based on distribution. MIC values from both mCBDE and BMD methods were classified using CLSI breakpoints: intermediate/susceptible (≤2 µg/mL) and resistant (>2 µg/mL). Diagnostic accuracy parameters were calculated using 2×2 confusion matrices, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Inter-method agreement was assessed using Cohen's kappa (κ) with 95% confidence intervals, where κ values of 0.61-0.80 indicate substantial agreement and >0.80 represent almost perfect agreement. Error analysis included major errors (MEs: false resistance) and very major errors (VMEs: false susceptibility), with acceptable rates of <10% and <3% respectively. Categorical agreement ≥90% was considered acceptable performance. Statistical significance was set at $p<0.05$.

RESULT
A total of 120 non-duplicate multidrug-resistant (MDR) *Enterobacterales* isolates were included in the study. These were obtained from a variety of clinical specimens submitted to the microbiology laboratory during the study period.

Table 1. Distribution of clinical specimens (N = 120)

Sample type	Number (%)
Pus	38 (31.7)
Urine	32 (26.7)

Endotracheal aspirate	21 (17.5)
Blood	12 (10.0)
Sterile body fluids	9 (7.5)
Sputum	8 (6.7)
Total	**120 (100)**

The distribution of sample types is summarized in **Table 1**. The highest number of isolates were recovered from pus samples (n = 38; 31.7%), followed by urine (n = 32; 26.7%) and endotracheal aspirates (n = 21; 17.5%). Blood cultures yielded 12 isolates (10.0%), sterile body fluids accounted for 9 isolates (7.5%), and sputum samples contributed 8 isolates (6.7%). This table depicts the origin of MDR *Enterobacterales* isolates. Pus and urine were the predominant sources, together accounting for more than half of the total isolates.

Table 2. Distribution of MDR *Enterobacterales* species (N = 120)

Species	**Number (%)**
Klebsiella pneumoniae	64 (53.3)
Escherichia coli	26 (21.7)
Enterobacter cloacae	19 (15.8)
Citrobacter freundii	11 (9.2)
Total	**120 (100)**

Species identification revealed that *Klebsiella pneumoniae* was the most frequently encountered organism, accounting for 64 isolates (53.3%). *Escherichia coli* represented 26 isolates (21.7%), *Enterobacter cloacae* 19 isolates (15.8%), and *Citrobacter freundii* 11 isolates (9.2%) (Table 2). The table illustrates species distribution among the isolates, with *K. pneumoniae* being the dominant pathogen, followed by *E. coli*

Table 3. MIC distribution (%) by method (N = 120)

MIC (µg/mL)	**BMD (%)**	**mCBDE (%)**
≤1	98 (81.6%)	97 (80.8%)
2	8 (6.7%)	5 (4.2%)
4	9 (7.5%)	9 (7.5%)
>4	5 (4.2%)	9 (7.5%)
Total	**100**	**100**

MIC values for colistin, determined by both the reference BMD method and the mCBDE method, are shown in **Table 3**. The distribution of MIC values determined by the reference BMD method showed that the majority of isolates, 98 out of 120 (81.6%), had MICs ≤1 µg/mL, indicating susceptibility to colistin. MICs of 2 µg/mL, 4 µg/mL, and >4 µg/mL were observed in 6.7%, 7.5%, and 4.2% of isolates, respectively. Using the mCBDE method, 97 isolates (80.8%) were reported with MICs ≤1 µg/mL, while MICs of 2 µg/mL, 4 µg/mL, and >4 µg/mL were observed in 4.2%, 7.5%, and 7.5% of isolates, respectively.

Table 4. Agreement between mCBDE and BMD Methods for Colistin Susceptibility Categorization (N = 120)

	mCBDE		**Total**	**Kappa Statistic (CI), p-value**
MIC BY MBD	**Resistant**	**Susceptible**		
Resistant	11 (61.1%)	3 (2.9%)	14 (11.7%)	κ = 0.64 (95% CI: 0.436 - 0.845), p < 0.001
Susceptible	7 (38.9%)	99 (97.1%)	106 (88.3%)	

A total of 120 MDR Enterobacterales isolates were tested using both the reference BMD and mCBDE methods. Among the 14 isolates identified as resistant by BMD, 11 (61.1%) were also reported resistant by mCBDE, while 3 (2.9%) were falsely identified as susceptible (Very Major Errors). Of the 106 isolates found susceptible by BMD, 99 (97.1%) were correctly identified as susceptible by mCBDE, and 7 (38.9%) were incorrectly classified as resistant (Major Errors).

Cohen's κ was run to determine if there was agreement between the two methods in categorizing isolates as colistin susceptible or resistant. Thus, the observed κ value of 0.64 It suggests that the mCBDE method shows a there was substantial agreement with the BMD method, supporting its potential utility as a cost-effective alternative for colistin susceptibility testing in clinical microbiology laboratories.

DISCUSSION

Colistin is the last line of antibiotic for carbapenem-resistant Gram-negative bacteria like *K. pneumoniae, E. coli, A. baumanii, P. aeruginosa* and *Enterobacteriaceae*. Colistin resistance develops by genetic alterations that modify the bacterial cell membrane or through the acquisition of plasmid-mediated resistance determinants such as the mcr-1 gene,

hence constraining treatment alternatives.[8] Reliable identification and reporting of its susceptibility pattern is of utmost importance for administering colistin in healthcare settings.[4,9] Presently, the development of an efficient, quick, cost-effective, and user-friendly diagnostic tool for detecting colistin resistance in standard microbiology laboratories remains a challenge, particularly in underprivileged countries.[10] The present study was conducted a assess the effectiveness of a modified microplate-based mCBDE protocol for colistin susceptibility testing in MDR *Enterobacterales* clinical isolates when compared to BMD method which is considered the gold standard.

The predominance of pus (31.7%) and urine (26.7%) specimens in our cohort mirrors the findings of Chauhan et al., who reported purulent exudates (35.1%) and urine (18.5%) as the leading sources of MDR Gram-negative isolates in a tertiary-care setting, underscoring the burden of surgical site and urinary tract infections in driving antimicrobial resistance.[11] Similarly, Rajeswari et al. observed that purulent exudates (35.1%) and blood (20.3%) were the most common sources of carbapenem-resistant Enterobacterales, highlighting the clinical significance of wound and bloodstream infections.[12]

Our recovery of 17.5% of isolates from endotracheal aspirates and 6.7% from sputum aligns closely with the 13% and 10% rates of endotracheal aspirates and bronchoalveolar lavage fluids reported by Rout et al., who documented a high prevalence of ventilator-associated and hospital-acquired respiratory infections due to MDR organisms.[9] The proportion of blood culture isolates in our study (10.0%) is somewhat lower than the 23% reported by Rout et al., but comparable to the 10.0–16.7% range noted by Kazaz et al. in their analysis of Klebsiella pneumoniae bacteremia.[6,9]

Sterile body fluids accounted for 7.5% of our isolates, a figure similar to the 9.2% of bronchoalveolar and pleural fluid isolates reported by Rajeswari et al, and the 6% from tissue biopsies and pleural fluids noted by Rout et al. (5% pleural fluid).[9,12] Our lower recovery from sputum (6.7%) compared with Rout et al.'s 13% endotracheal aspirate rate may reflect differences in sampling practices and patient populations, particularly the proportion of mechanically ventilated patients.[9] Collectively, these comparisons indicate that pus and urine remain the principal reservoirs for MDR Enterobacterales across diverse clinical contexts, as consistently observed by Chauhan et al. and Rajeswari et al.[11,12] The substantial contribution of respiratory samples documented by Rout et al. and Kazaz et al. further emphasizes the critical need for targeted infection control measures in intensive care units.[6,9] Our findings, in concordance with these prior studies, reinforce the importance of comprehensive surveillance and prompt susceptibility testing, ideally using reliable, high-throughput methods such as the modified CBDE to guide effective antimicrobial therapy in patients with invasive MDR infections.

The predominance of Klebsiella pneumoniae (53.3%) in our isolate collection closely parallels the findings of Nirmal et al., who reported K. pneumoniae as the leading carbapenem-resistant Gram-negative blood culture isolate (60.4%) in their cohort of 106 strains.[7] Similarly, Rout et al. observed K. pneumoniae as the second most common species (28.1%) among 6,013 carbapenem-resistant isolates, with Escherichia coli accounting for 37.5% and Enterobacter cloacae for 10.6%.[9] Although our proportion of E. coli (21.7%) is lower than that in Rout et al., it remains the second most frequent pathogen, consistent with global trends in MDR Enterobacterales infections.[9] The 15.8% prevalence of E. cloacae in our study exceeds the 10.6% reported by Rout et al., suggesting variability in local epidemiology and specimen sources.[9] Our Citrobacter freundii rate (9.2%) aligns with the lower frequency of non-Klebsiella Enterobacterales species seen across multiple studies, including Germ et al.'s evaluation of Colistin AST methods, where Citrobacter spp. comprised only 7.6% of isolates tested.[13] These comparisons underscore K. pneumoniae's dominant role in MDR Enterobacterales infections, while highlighting institutional differences in species distribution that may inform targeted surveillance and antimicrobial stewardship strategies.

The MIC distributions obtained by BMD in our study, where 81.6% of isolates exhibited MICs ≤1 µg/mL, align closely with Kar et al., who reported 88.8% of 160 CRE isolates as intermediate susceptible (MICs ≤2 µg/mL) by BMD, underscoring the predominance of isolates at or below the CLSI breakpoint.[1] Similarly, Antony et al. found that 100% of their 49 CRKP isolates had identical BMD and CBDE MICs, with 96.1% of isolates showing MICs ≤1 µg/mL, reflecting high concordance at low MIC ranges.[14] In contrast, Ülker et al. observed higher MIC50 and MIC90 values (16 µg/mL and 128 µg/mL, respectively) in a cohort of 215 Enterobacterales, indicating a greater burden of high-level resistance in their setting.[15]

When comparing mCBDE MICs, our 80.8% of isolates with MICs ≤1 µg/mL and the slight increase in isolates with MICs >4 µg/mL (7.5%) mirror the trends reported by Kazaz et al., who documented 50% susceptibility by both methods but noted occasional upward MIC shifts near breakpoints, contributing to bias rates of 10.8% for CBDE.[6] Kar et al. also reported a small VME rate (5.5%) and ME rate (2.1%) for mCBDE, largely involving isolates with MICs of 4–8 µg/mL, highlighting challenges in detecting borderline resistance.[1] Collectively, these comparisons confirm that mCBDE reliably mirrors BMD for isolates with low MICs but may overestimate resistance in those with MICs at or above the clinical breakpoint, consistent with previous evaluations.

The error rates and agreement observed in our study closely mirror prior evaluations of mCBDE. Our very major error (VME) rate of 2.9% is comparable to Kar et al., who reported a VME of 5.5% among 160 CRE isolates when comparing mCBDE to BMD, predominantly involving isolates with MICs at 4–8 µg/mL.[1] Likewise, our major error (ME) rate of 6.6% (7/106) approximates the 2.1% reported by Kar et al. and the 8.6% ME observed by Kazaz et al. in their analysis of 116 MDR K. pneumoniae isolates.[1,6] The Cohen's κ of 0.64 in our study indicates substantial agreement, aligning with the substantial-to-almost-perfect κ values reported in other comparative studies. For instance, Simner et al. documented κ values ≥0.80 for CBDE versus BMD across multiple sites, whereas Kar et al. noted κ indicative of strong agreement for mCBDE. Collectively, these findings support the diagnostic reliability of mCBDE as a cost-effective alternative to BMD, particularly for isolates with clear susceptibility or resistance, while reinforcing the need for confirmatory BMD testing in borderline cases.[1,16]

Notably, broth disk elution (BDE) methods have also been successfully extended to other antibiotic combinations. For example, a recent study evaluated the in vitro activity of aztreonam combined with ceftazidime/avibactam against 204 metallo-β-lactamase–producing *Enterobacterales* using both 5 mL and 2 mL BDE formats, with reference broth microdilution. The BDE-5 mL assay achieved categorical agreement of 99.5% for the 6/6/4 mg/L aztreonam/ceftazidime/avibactam tube and 100% for the 12/6/4 mg/L tube, with negligible very major errors (0%) and only 0.5% major errors. Although the 2 mL format exhibited higher very major error rates at raised concentrations, the 5 mL BDE-12/6/4 mg/L tube demonstrated robust performance, reinforcing that microplate-based disc elution protocols can be reliably adapted for diverse antibiotic regimens in routine clinical laboratories.[17] In light of similar efforts beyond *Enterobacterales*, Sharma et al. evaluated the CBDE method for Acinetobacter baumannii, testing 125 isolates (100 susceptible and 25 resistant) against the BMD reference. They reported an essential agreement of 97.6%, categorical agreement of 98.4%, sensitivity of 100%, and specificity of 98.4%, with a major error rate of 1.6% and no very major errors. These results suggest that CBDE may be a viable option for colistin susceptibility testing in A. baumannii, particularly in resource-limited settings where rapid, affordable methods are critically needed.[18]

The present study demonstrates that the microplate-based mCBDE method offers a practical and resource-sparing approach to colistin susceptibility testing, yet several limitations warrant consideration. First, the study's sample size of 120 isolates while adequate for initial validation may not capture the full spectrum of resistance mechanisms, particularly rare mcr-mediated variants. Second, borderline MIC values (2–4 µg/mL) accounted for most discordant results, reflecting the method's reduced precision near clinical breakpoints and underscoring the need for confirmatory BMD in equivocal cases. Third, reliance on commercial colistin discs introduces potential variability in elution efficiency, which could affect reproducibility across laboratories. Finally, the single-center design and focus on Enterobacterales restrict generalizability to other Gram-negative pathogens.

Despite these constraints, mCBDE offers notable advantages. The microplate format significantly reduces reagent volumes and hands-on time compared with traditional BMD and CBDE protocols, enabling high throughput and streamlined workflow. The method achieved substantial categorical agreement (90.8%) and a Cohen's κ of 0.64, indicating strong concordance with BMD. Very major and major error rates remained within acceptable thresholds (<3% and <7%, respectively), validating mCBDE's diagnostic reliability for clear-cut susceptible or resistant isolates. The cost savings from using discs instead of colistin powder and the minimal equipment requirements make mCBDE especially appealing for resource-limited settings.

Clinically, mCBDE can enhance antimicrobial stewardship by accelerating access to accurate colistin susceptibility results, thereby guiding timely initiation or de-escalation of therapy. This is critical for managing severe infections caused by multidrug-resistant Enterobacterales, where colistin remains a last-line agent. Adoption of mCBDE could standardize testing practices across diverse laboratory environments, support epidemiological surveillance of colistin resistance, and ultimately improve patient outcomes by informing targeted, evidence-based antimicrobial interventions.

CONCLUSION

In conclusion, the microplate-based mCBDE method demonstrated substantial agreement (κ=0.64) with the reference broth microdilution, achieving 90.8% categorical agreement, 2.9% very major errors, and 6.6% major errors. Its reduced reagent use, streamlined workflow, and cost-effectiveness make mCBDE a practical alternative for routine colistin susceptibility testing in multidrug-resistant *Enterobacterales*. While confirmatory BMD is advised for borderline MICs, mCBDE's high accuracy supports its implementation in clinical laboratories, especially those with limited resources.

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