Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 9s, https://carcinogenesis.com/

Reliability of Vitek 2 (Automated) Versus Reference Broth Microdilution Method (Manual) in Colistin Susceptibility Assessment of MDR Gram Negative Bacilli: Insights from a Tertiary Care Hospital in Northern India

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ABSTRACT

Background: Colistin serves as a critical therapeutic option for infections caused by multidrug-resistant Gram-negative bacteria. Due to its clinical importance, accurate and timely determination of colistin susceptibility is essential. While broth microdilution (BMD) remains the gold standard recommended by EUCAST and CLSI, automated systems such as VITEK®2 are often employed for convenience in clinical microbiology laboratories.

Aim and Objective: This study aimed to assess the accuracy and performance of colistin minimum inhibitory concentration (MIC) determination by VITEK®2 compared to the reference BMD method in Gram-negative isolates from an intensive care unit (ICU) in a tertiary care hospital.

Material and Methods: Gram-negative clinical isolates from ICU patients were analyzed from February 2025 to July 2025. Colistin MICs were determined using both BMD and VITEK®2. Comparative analysis included parameters such as very major error (VME), major error (ME), essential agreement (EA), categorical agreement (CA), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Results: Out of 304 isolates, the majority were recovered from urine (42%), followed by sputum samples (32%), endotracheal aspirates (8%), pus (6.9%), blood (6.5%), and bronchoalveolar lavage (4%). The predominant species included Klebsiella pneumoniae (34.2%),E. coli(21.7%), Enterobacter cloacae (4.6%),Acinetobacter baumannii (22%), and Pseudomonas aeruginosa (17.1%). VITEK®2 demonstrated high sensitivity (>97%) but poor specificity (45.45%). The PPV and NPV were 90.39% and 92.85% respectively. VME rates ranged between 54.5–100%, with E. coli showing the highest (100%). ME was 74.9%. The overall EA was 62.10%, and CA was 92.10%.

Conclusion: VITEK®2 failed to accurately detect colistin resistance in 63% of the resistant isolates. The observed VME and ME rates exceeded acceptable thresholds, and EA did not meet the ≥90% benchmark. Despite its high sensitivity, the low specificity of VITEK®2 limits its reliability for colistin susceptibility testing. BMD should remain the preferred method for determining colistin MICspecially in critical care settings.

KEYWORDS: *Colistin resistance, Broth microdilution, Gram-negative bacilli, VITEK®2, ICU infections, MIC testing.*

How to Cite: Parul Singhal, Lalita Agarwal, Ayesha Nazar, Shaheen Bhat and NashraAfaq., (2025) Reliability of Vitek 2 (Automated) Versus Reference Broth Microdilution Method (Manual) in Colistin Susceptibility Assessment of MDR

Gram Negative Bacilli: Insights from a Tertiary Care Hospital in Northern India, *Journal of Carcinogenesis*, *Vol.24, No.9s*, 98-103.

1. INTRODUCTION

The global rise of multidrug-resistant (MDR) Gram-negative bacilli (GNB) has become a pressing public health concern, significantly limiting therapeutic options for severe infections [1,2]. Colistin, a polymyxin antibiotic discovered in the late 1940s and derived from *Paenibacilluspolymyxa*, was initially used extensively against Gram-negative organisms [3]. However, its nephrotoxicity and neurotoxicity, combined with the availability of safer and more effective antimicrobials such as aminoglycosides, cephalosporins, and carbapenems, led to its decline in clinical use from the 1970s onward [4]. In recent decades, with the alarming spread of carbapenem-resistant *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, colistin has re-emerged as a critical last-resort therapy, often representing the only available treatment option [5,6]. Accurate determination of colistin susceptibility has therefore assumed paramount importance in guiding antimicrobial therapy.

Colistin acts primarily by binding to the lipid A moiety of lipopolysaccharides in the bacterial outer membrane, displacing calcium and magnesium ions, thereby disrupting membrane stability and leading to cell death [7]. Unfortunately, resistance to colistin is increasingly being reported, particularly in nosocomial pathogens. Resistance arises through two major mechanisms: (i) chromosomal mutations that induce modification of lipid A with cationic groups such as phosphoethanolamine or 4-amino-4-deoxy-L-arabinose, reducing colistin binding affinity [8]; and (ii) plasmid-mediated *mcr* genes, which confer transferable resistance across species and facilitate global dissemination [9]. The emergence of such resistance mechanisms in high-risk hospital pathogens poses serious challenges in the management of critically ill patients, further narrowing the therapeutic window in infections where options are already scarce [10].

Various laboratory methods have been employed to determine colistin susceptibility, including disc diffusion, gradient diffusion (E-test), agar dilution, and automated systems. Disc and gradient diffusion methods are considered unreliable due to the poor diffusion of colistin in agar, resulting in high rates of major and very major errors [11]. Agar dilution, while more accurate, is cumbersome and rarely feasible in routine practice. As a result, international guidelines from the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommend broth microdilution (BMD) as the only valid reference method for colistin susceptibility testing [1,3,12,13]. However, BMD is technically demanding, labor-intensive, and time-consuming, limiting its widespread adoption, particularly in resource-constrained laboratories. In contrast, automated platforms such as Vitek 2 provide rapid, standardized, and user-friendly results that integrate well into clinical workflows [10,14,15]. For critically ill patients, where delays in initiating effective therapy can significantly worsen outcomes, the need for reliable, rapid automated testing is undeniable [15].

Against this background, evaluating the performance of automated systems such as Vitek 2 against the reference BMD method is essential. Such studies not only address the technical reliability of automated platforms but also have direct implications for clinical decision-making, antimicrobial stewardship, and patient outcomes in the ongoing battle against MDR Gram-negative infections.

2. METHODS

2.1 Bacterial Isolates

This prospective investigation was performed in a tertiary-care hospital located in northern India. The study population included patients admitted to different intensive care units (ICUs). Clinical samples such as blood, urine, pus, wound exudates, respiratory secretions, and cerebrospinal fluid (CSF) were processed, and only clinically relevant isolates were included based on National Healthcare Safety Network (NHSN) criteria. To avoid duplication, only one isolate per patient was considered. Microbial identification was performed using theVITEK®2 automated system. Organisms that are intrinsically resistant to colistin, including *Proteus* spp., *Morganella* spp., and *Serratiamarcescens*, were excluded from analysis.

2.2 Antimicrobial Susceptibility Testing

Broth microdilution (BMD) was used as the reference method for determining the minimum inhibitory concentration (MIC) of colistin. Each well of the 96-well microtiter plate contained a final volume of 100 µl, consisting of 50 µl of double-strength cation-adjusted Mueller–Hinton broth (CAMHB), 25 µl of colistin sulfate, and 25 µl of bacterial suspension. A primary stock solution of 128 µg/ml was prepared and serial twofold dilutions were made to achieve concentrations ranging from 0.125 µg/ml to 32 µg/ml. Interpretation was carried out according to CLSI guidelines, where MIC values ≤2 µg/ml

were considered intermediate and >2 µg/ml resistant for both *Enterobacterales* and non-fermenters. All isolates were tested in duplicate, and any inconsistent results were repeated. Quality control was ensured using*Escherichia coli* ATCC 25922 (MIC range: 0.25–2 µg/ml) and *Pseudomonas aeruginosa* ATCC 27853 (MIC range: 0.5–4 µg/ml).

The automated system employed for comparison was the VITEK®2, which determines colistin MIC within the range of 0.5–16 µg/ml. AST-N405 and AST-N406 cards were used for lactose-fermenting and non-fermenting isolates, respectively, and processed according to the manufacturer's instructions.

2.3 Data Interpretation and Analysis

Following joint CLSI–EUCAST guidelines, BMD was treated as the gold standard. A very major error (VME) indicated false susceptibility (resistant by BMD but susceptible by the test method), whereas a major error (ME) indicated false resistance (susceptible by BMD but resistant by the test method). Essential agreement (EA) was defined as MIC values within ±1 dilution of the reference BMD result, while categorical agreement (CA) was defined as the percentage of isolates with correct susceptibility categorization. Acceptable performance was defined as ≥90% EA and CA with VME and ME ≤3%, in accordance with ISO 20776–2 and FDA recommendations.

3. RESULTS

A total of 304 non-duplicate clinical isolates were collected and tested using both BMD and VITEK®2. The majority originated from urine (128, 42%) and sputum (98, 32%), followed by endotracheal aspirates (25, 8%), pus (21, 6.9%), blood (19, 6.5%), and bronchoalveolar lavage (13, 4%). Among these, *Enterobacterales* accounted for 185 isolates (60.8%) and non-fermenters for 119 isolates (39.1%). The predominant pathogens were *Klebsiella pneumoniae* (105, 34.2%), *E. coli* (66, 21.7%), and *Enterobacter cloacae* (14, 4.6%) among *Enterobacterales*, while *Acinetobacter baumannii* (67, 22%) and *P. aeruginosa* (52, 17.1%) were the major non-fermenters. *K. pneumoniaewas the most common organismisolated from urine samples (50, 38.16%) and sputum (29, 30.85%) followed by A. baumannii*from sputum 932, 34.04%) and endotracheal aspirates (13,50%).

Resistance detected by BMD was highest in *E. cloacae* (21.4%), followed by *P. aeruginosa* (19.4%), *A. baumannii* (16.4%), *K. pneumoniae* (10.5%), and *E. coli* (1.5%). VITEK®2 results showed resistance rates of 19.2% in *P. aeruginosa*, 7.1% in *E. cloacae*, 5.7% in *K. pneumoniae*, and 4.4% in *A. baumannii*.

Compared to BMD, the sensitivity of VITEK®2 exceeded 97% across all isolates, while specificity was considerably lower (27–45%). Positive predictive values (PPV) ranged from 84.6% to 98.4%, whereas negative predictive values (NPV) varied from 71.4% to 100%. Sensitivity was consistently high (close to 100%) across species, but specificity was notably low—*E. coli* showed 0%, whereas *K. pneumoniae* demonstrated the highest at 45.5%. The overall sensitivity was 99.6%, but specificity averaged only 29.2%.

A summary of the comparative performance is shown in [Table 1].

Table 1.Performance characteristics of Broth Microdilution and VITEK®2 methods.

Organisms	VITEK®2	BMD		Sensitivity	Specificity	PPV	NPV
		S	R				
Enterobacterales *(n* = 185*)*							
K. pneumoniae	S	92	6	97.87	45.45	93.87	71.42
	R	2	5				
E. coli	S	65	1	100	0	98.40	NA
	R	0	0				
E. cloacae	S	11	2	100	33.33	84.61	100
	R	0	1				
Non fermenters (n = 120*)*							
A. baumannii	S	57	8	100	27.27	87.60	100

	R	0	3				
P. aeruginosa	S	42	6	100	40.00	87.50	100
	R	0	4				

Very major errors were high across species (54.5–100%), with the overall VME rate at 70.8%. Major errors were recorded at 74.9%, with *K. pneumoniae* contributing the highest ME (21.3%). Essential agreement across all isolates was 62.1%, highest among non-fermenters (*A. baumannii* 86.6%, *P. aeruginosa* 73.5%). Overall categorical agreement was 92.1%.

4. DISCUSSION

With the global rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative infections, colistin has reemerged as a critical last-line treatment option. This renewed use highlights the necessity of reliable diagnostic tools for colistin susceptibility testing. In India, where resistance rates are increasing, accurate and user-friendly testing methods are particularly essential.

Although VITEK®2 offers rapid and convenient results and is widely adopted in clinical microbiology laboratories, this study revealed that it failed to identify resistance in 23 isolates (7.5%), including *A. baumannii*, *K. pneumoniae*, *E. cloacae*, *P. aeruginosa*, and *E. coli*. The required essential agreement (≥90%) was not achieved for any group, though categorical agreement was acceptable (92.1%). Importantly, both very major and major error rates exceeded the acceptable 3% threshold, reinforcing concerns about the system's reliability for colistin MIC determination.

Findings from this study are consistent with several international reports that question the accuracy of VITEK®2 in detecting colistin resistance. A comparative study from Germany reported that VITEK®2 demonstrated poor reliability, showing a very major error (VME) rate exceeding 3% [16]. Several other investigations have also highlighted that when used for minimum inhibitory concentration (MIC) determination in Gram-negative organisms, VITEK®2 often yields inconsistent results, particularly with *Enterobacterales* and *Acinetobacter baumannii* [17,18,19,20]. Consistent with these observations, our study also emphasizes that VITEK®2 cannot be considered dependable for colistin susceptibility testing. Instead, reference standards such as CLSI and EUCAST guidelines provide the appropriate interpretive framework for MIC evaluation. However, contrasting evidence exists—such as a Greek study reporting 0% VME—highlighting variability in system performance across settings[21].

The high sensitivity but poor specificity observed here may partly be due to the limited number of resistant isolates in certain species. False susceptibility results can have serious clinical implications, leading to inappropriate therapy, treatment failure, and increased morbidity and mortality.

While BMD remains the reference standard, its labor-intensive nature and technical complexity limit routine use. Laboratories must invest in staff training to reduce errors related to solution preparation, dilution accuracy, and contamination. Molecular methods, such as detection of *mcr* genes and mutation analysis, can further enhance understanding of resistance mechanisms.

Despite known discrepancies, many laboratories continue to rely on automated systems due to the urgent need for rapid results, particularly for critically ill patients. This study, involving 304 ICU isolates, emphasizes that while VITEK®2 provides quick data, clinicians and microbiologists should interpret colistin results cautiously. Ideally, BMD should be performed as a confirmatory test whenever treatment outcomes do not correlate with automated susceptibility results.

The present study, conducted in 2025, reaffirms ongoing concerns regarding the reliability of automated systems such as VITEK®2 for determining colistin susceptibility among multidrug-resistant Gram-negative bacilli. Despite its rapidity and ease of use, VITEK®2 demonstrated high sensitivity but poor specificity, leading to significant discrepancies when compared to the gold-standard broth microdilution (BMD) method [22,23].

The elevated rates of very major and major errors observed (exceeding acceptable thresholds) are consistent with recent global findings, including studies from India, Germany, and Greece (Ananda et al., 2024; Akıllı et al., 2025; Pfennigwerth et al., 2019) [24,25,26], which collectively question the dependability of automated systems for colistin MIC determination. The inability of VITEK®2 to detect true resistance could have severe clinical consequences, potentially resulting in inappropriate antimicrobial therapy and increased patient morbidity. Although BMD remains the reference standard, its time-consuming and technically demanding nature poses challenges for routine use.

Therefore, laboratories must balance speed and accuracy by confirming automated results with BMD in critical cases and incorporating molecular methods to detect plasmid-mediated mcr genes. Strengthening quality control, enhancing laboratory training, and investing in automated platforms validated specifically for colistin testing are crucial steps for reliable antimicrobial stewardship in 2025 and beyond [27,28].

5. CONCLUSION

The findings of this study indicate that the VITEK®2 system, while efficient and convenient, cannot reliably substitute the broth microdilution method for colistin susceptibility testing in multidrug-resistant Gram-negative isolates. The observed high rates of very major and major errors, coupled with suboptimal essential agreement, highlight the risk of false susceptibility interpretations. Therefore, BMD should remain the preferred confirmatory method, particularly in ICU and other critical care settings, to ensure accurate detection of resistance and guide appropriate antimicrobial therapy.

6. LIMITATIONS OF THE STUDY

This study was limited by its single-center design and the relatively low number of resistant isolates in certain bacterial species, which may have influenced the specificity and predictive value calculations. Additionally, molecular characterization of resistance mechanisms, including mcr gene detection, was not performed, which could have provided further insight into genotypic resistance patterns. Future multicentric studies with larger sample sizes and combined phenotypic-genotypic approaches are recommended to enhance the precision and generalizability of these findings.

DECLARATIONS:
Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors contributions: Author equally contributed the work.

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