International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Evaluation of VITEK versus Broth Microdilution for Determination of Colistin MIC in Carbapenem-Resistant Enterobacteriaceae from an Oncology Centre

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Abstract: ***Between June 2025 and May 2026, a study was conducted at an oncology hospital to explore how well the Vitek 2 Compact system measures the minimum inhibitory concentration (MIC) of Colistin compared to the broth microdilution (BMD) method in carbapenem-resistant Enterobacteriaceae (CRE) strains isolated from cancer patients. This research aimed to see if Vitek 2 Compact could reliably guide treatment decisions in this sensitive healthcare setting.The study included 59 CRE isolates from various infection sites, excluding those naturally resistant to Colistin. Most isolates were Escherichia coli (71%), with the rest being Klebsiella pneumoniae (29%). Identification and susceptibility testing were done using Vitek 2 Compact, while the BMD method was performed in the lab using MICROLATEST® kits, following EUCAST 2019 guidelines for interpreting Colistin susceptibility. Results showed a strong correlation between the two methods. When BMD was considered the gold standard, the essential agreement was 96.6%, and the categorical agreement reached 98.3%. However, one Klebsiella pneumoniae isolate showed a very major error, being classified differently by the two methods. Out of all isolates, only one was truly Colistin-resistant according to BMD. The study concluded that BMD remains a reliable and practical method for Colistin MIC determination, although its cost can be a limiting factor. Vitek 2 Compact showed high reliability in this study, but the small sample size means these findings are preliminary. Larger, multicenter studies are needed to confirm these results and to better understand how Colistin MIC breakpoints relate to clinical outcomes in oncology patients.***

Keywords: Broth micro dilution (BMD), Minimum inhibitory concentration (MIC).

1. Introduction

Cancer patients are immunosuppressed and therefore highly vulnerable to various infections. Infections caused by multi-drug resistant organisms (MDROs) are particularly dangerous for them, as improper treatment can be fatal. Increasingly, MDROs are being identified that are only susceptible to Colistin. Polymyxins, including Polymyxin B and Colistin, often serve as the last-resort antibiotics. Colistin remains a critical part of the last-line treatment for multi-drug resistant gram-negative bacilli (GNB), such as carbapenem-resistant strains. Currently, the CLSI-EUCAST joint recommendation is to use broth microdilution (BMD) as the reference method for testing Colistin susceptibility (1). Colistin has proven to be a powerful antibiotic in the fight against infections caused by bacteria such as *Pseudomonas*, *Klebsiella*, and *Escherichia*. It is especially effective against many Gram-negative bacteria that have developed resistance to multiple drugs. Due to the increasing challenge posed by these resistant bacteria, colistin is now used as a last-resort treatment option. However, as the use of colistin has grown, so has the concern over rising resistance among Gram-negative bacteria, highlighting the ongoing struggle to manage these serious infections. (2). Automated systems are increasingly used for quick identification and antimicrobial susceptibility testing in clinical laboratories. Recently, researchers Tan and Ng evaluated the performance of the VITEK 2 system's colistin susceptibility test by comparing it to the traditional agar dilution method. Their findings revealed that the VITEK 2 test was unreliable for accurately determining colistin susceptibility. This raises important considerations for laboratories relying on automated methods to ensure correct antibiotic treatment decisions. (3). It's clear that accurate and comprehensive antimicrobial susceptibility testing (AST) is essential for colistin due to its critical role. However, many AST methods show inconsistent results. In response, following initiatives by EUCAST and CLSI to establish standardized polymyxin breakpoints, the CLSI Working Group for Colistin Susceptibility Testing was formed to address these challenges and improve testing reliability. (4). When testing bacteria for colistin resistance, choosing the right method is crucial. Experts recommend the Broth Microdilution technique as the best approach for accurate colistin antimicrobial susceptibility testing (AST). This is because diffusion methods, like disc diffusion, are not reliable due to the large size of the colistin molecule, which prevents it from spreading effectively in these tests. However, some studies show that gradient methods, which are similar to diffusion, can produce very different minimum inhibitory concentration (MIC) results, leading to inconsistencies. This highlights the importance of using standardized and appropriate techniques like Broth Microdilution to ensure trustworthy and consistent results in colistin testing. (5). Recently, a joint working group from EUCAST and CLSI reviewed the challenges faced by two types of colistin gradient tests. These tests, one manufactured by bioMérieux and the other by Liofilchem (included in the CLSI guidelines), have both undergone careful verification. This collaboration highlights the ongoing efforts to ensure reliable and accurate antimicrobial testing methods. (6). Colistin has traditionally been reported using automated systems like VITEK for many years. However, the CLSI 2026

guidelines now approve only the broth microdilution method for determining colistin MIC (Minimum Inhibitory Concentration).

The two main issues affecting colistin in MIC trays are its tendency to attach to surfaces like polystyrene, which can reduce the effective colistin concentration. This makes accurate colistin susceptibility testing a significant challenge for clinical labs. Additionally, it is hard to find a commercial reference standard procedure to confirm equivalency of tests. Although several studies have used the standard agar dilution (AD) method, the CLSI has seldom used AD as a reference in recent years. Instead, broth microdilution (BMD) is the primary reference technique for colistin MIC testing. However, colistin's strong attachment to the plastic in BMD panels can notably affect results, especially at low antibiotic concentrations. (8)

2. Materials and Method

Study Setup:

- Location: Exclusive cancer center with 170 beds, 70 functional beds
- Duration: 1 year (June 2025 to May 2026)

Inclusion Criteria:

- Samples from all cancer patients with suspected infections hospitalized during the study period
- Screening of high-risk cases (e.g., Leukemia, Colonic malignancy) for CRE infection before starting therapy (chemotherapy or surgery)

Samples and data collection:

The study focused on carbapenem-resistant Enterobacteriaceae, a challenging group of bacteria isolated from various clinical samples. These samples were collected from cancer patients suspected of infections at different sites. To ensure accuracy, only non-duplicate clinical specimens were included. Data and specimens came from both inpatient wards and the outpatient department of the oncology care hospital, Balco Medical Centre in Raipur, during the period from June 2025 to May 2026. The clinical samples analyzed in this research included wound swabs, oral swabs, respiratory secretions, and blood samples. Alongside the specimens, clinical and demographic information was gathered from patients' electronic medical records, providing a comprehensive context for understanding the infections and their resistance patterns.

Refer to Table No 1. For the Distribution of isolates from various samples.

Table 1: Distribution of isolates from various samples

Culture samples	Pus	Blood	Urine	Body fluid	Sputum	Total
Total received	283	105	78	48	39	553
Positive isolates	177	21	29	9	13	249
Negative Isolates	106	84	49	39	26	304
Gram Negative isolates#	112(71)	12(10)	21(19)	9(8)	6(4)	160(112)
MDRO isolates	27	7	16	7	2	59

Bacterial isolations and susceptibility:

In a clinical microbiology lab, samples are carefully prepared and cultured to detect bacterial infections. Initially, the samples are placed on three types of agar plates: MacConkey Agar, Tryptose Blood Agar with 5% sheep blood, and Chocolate Agar. These plates are incubated at 37℃ for 24 hours to allow any bacteria present to grow.

If no bacterial growth is observed after the first day, the samples are re-incubated for another 24 hours to ensure no slow-growing bacteria are missed. When bacteria are detected, colonies are isolated for further identification and antibiotic susceptibility testing using the Vitek 2 compact system.

Special attention is given to bacteria that show resistance to carbapenem antibiotics, known as Carbapenem-resistant Enterobacteriaceae. These strains undergo additional testing for susceptibility to colistin, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (7). This includes the colistin broth microdilution (BMD) test and using the Vitek 2 susceptibility card N405, which specifically assesses colistin resistance according to manufacturer instructions. The minimum inhibitory concentrations (MICs) of colistin used to determine resistance are 0.5, 1.0, 2.0, and 4.0 µg/ml, with an MIC of 4.0 µg/ml or higher indicating resistance. This thorough process ensures precise detection and characterization of resistant bacterial strains, guiding effective treatment decisions.

Refer to Table No. 2- MIC distribution in MDROs by BMD is as follows.

Table 2: MIC distribution in MDROs by BMD is as follows.

Org	No. of isolates	MIC <= 0.25 mcg/ml		MIC 0.50mcg/ml		MIC 1.0mcg/ml		MIC 2.0mcg/ml		MIC 4.0mcg/ml	
		VTK	BMD	VTK	BMD	VTK	BMD	VTK	BMD	VTK	BMD
Eco	42	-	-	35	23	7	18	-	1	-	-
KPN	17	-	-	15	8	2	8	-	-	-	1

Broth Micro Dilution:

In our lab, we perform the Colistin broth microdilution (BMD) test using the MICROLATEST kit, which is marketed by Transasia in India. This test helps determine the minimum inhibitory concentration (MIC) of colistin against bacteria, guiding effective treatment decisions.

The MICROLATEST kit provides a range of colistin concentration cutoffs, specifically at 0.25, 0.5, 1.0, 2.0, 4.0,

8.0, and 16.0 mcg/ml. These values allow precise measurement of bacterial sensitivity or resistance to colistin, ensuring accurate and reliable results for clinical use.

By using this standardized method, our lab contributes to better antibiotic stewardship and patient care.

3. Result

A study tested a total of 59 CRE isolates, consisting mainly of Escherichia coli (71%, 42 out of 59) and *Klebsiella pneumoniae* (29%, 17 out of 59). Among these, a very major error was identified in one *Klebsiella pneumoniae* case, representing 1.69% of the isolates. Detailed discrepancies are provided in Table No. 3. When comparing the results of MIC versus BMD tests, 28.5% minor errors were observed in Escherichia coli isolates. For *Klebsiella pneumoniae*, there were 41.1% minor errors and 5.9% very major errors. These error rates are illustrated in Figure No. 1, highlighting the differences between minor and major errors in the testing process.

Table 3: Details of the discrepancy

Isolates	Mic by Vitek	Mic by BMD	Types of error
KPN blood (2)	0.50	1.0	Minor
Eco blood	0.50	1.0	Minor
KPN fluid (2)	0.50	1.0	Minor
Eco fluid	0.50	1.0	Minor
Eco et secretions	0.50	1.0	Minor
KPN pus	1	4	Very major error
KPN pus	0.50	1.0	Minor
Eco pus (5)	0.50	1.0	Minor
KPN urine (2)	0.50	1.0	Minor
Eco urine (4)	0.50	1.0	Minor

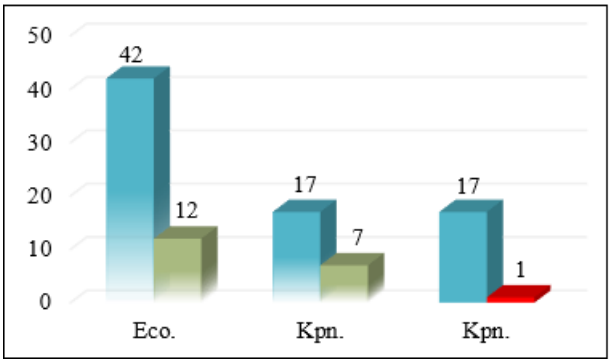

Figure 1: Minor and Major error

CRE isolates were obtained from various specimens as follows:

- **Pus:** 46% (27/59)
- **Urine:** 27% (16/59)
- **Sputum:** 3% (2/59)
- **Blood:** 12% (7/59)
- **Body fluid:** 12% (7/59)

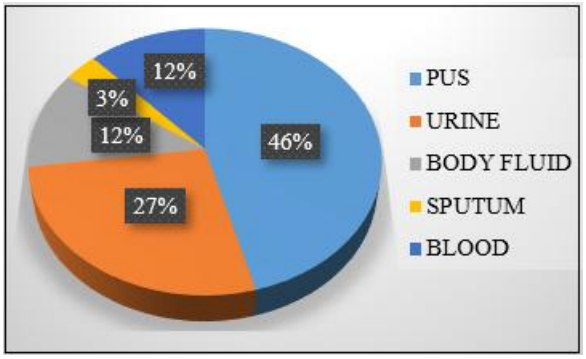

Figure 2: Isolate of CRE from different types of specimen in cancer patients

In this study, researchers isolated 59 carbapenem-resistant Enterobacteriaceae (CRE) samples from patients with various types of cancer. The breakdown of these isolates showed that the majority came from head and neck carcinoma patients, accounting for 32% (19 out of 59). Gastrointestinal carcinoma patients contributed 25% (15 isolates), while leukemia patients represented 17% (10 isolates). Patients with genitourinary carcinoma made up 12% (7 isolates), and breast carcinoma patients accounted for 14% (8 isolates). These findings highlight the presence of CRE across different cancer types, underscoring the importance of monitoring and managing infections in these vulnerable populations. For a visual representation, refer to Figure 3, which displays an isolate of CRE from cancer patients.

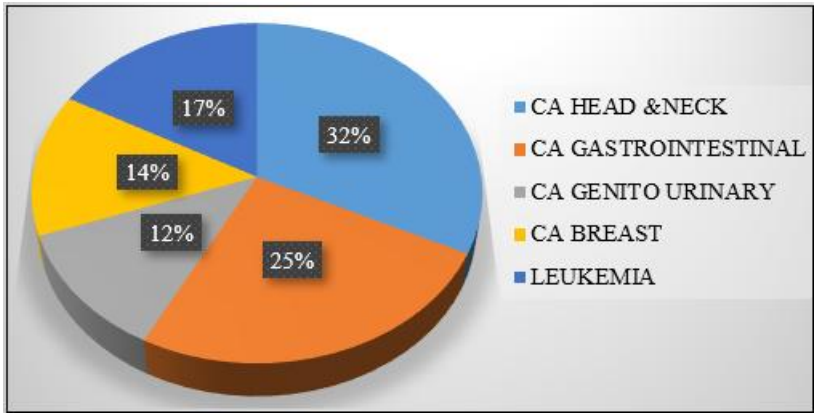

Figure 3: Isolate of CRE from different types of specimens in cancer patients

Data analysis:
In a recent evaluation of Colistin susceptibility testing, the Vitek 2 Compact system was compared to the reference method, Broth Microdilution (BMD). The results showed a high level of reliability, with an essential agreement of 96.6% and a categorical agreement of 98.3%. These findings highlight the Vitek 2 Compact as a dependable tool for accurately determining Colistin susceptibility, offering confidence to laboratories in their antimicrobial testing processes.

4. Discussion & Conclusion

The BMD (broth micro-dilution) method for determining the Colistin MIC is both reliable and user-friendly, making it a practical choice for accurate antimicrobial susceptibility testing (2).

In our study with the Vitek 2 Compact system, except for one isolate showing a very major error and nine minor errors, the broth micro-dilution (BMD) method should be used for correlation and to recheck all MDROs' Colistin MIC results.

For *Klebsiella pneumoniae*, out of 40 isolates, only one showed discrepancy in MIC values where Vitek reported an MIC of ≥16 mcg/ml. We infer that for *Klebsiella pneumoniae*, reconfirmation by BMD is necessary only when the MIC is ≥16 mcg/ml. However, a larger number of isolates need to be studied to confirm this inference (Shaikh S. et al., 2019).

Overall, the reliability of Vitek 2 Compact was high when compared to BMD as the reference method in our study, but the small sample size limits definitive conclusions. Larger multicentric studies are needed to validate these findings.

However, it is pure that the occurrence of colistin resistance might be suggestively higher among MDR isolates, for which colistin might be used in conduct. In particular, in our study, a high rate (45%) of colistin resistance was detected amongst the 20 MDR *klebsiella pneumoniae* isolates tested, an organism for which investigation studies have noted only 1.5% resistance among all *klebsiella pneumoniae* isolates examined (11).

This study's results might have a noteworthy impact on clinical research evaluating patient outcome, which is usually reliant on the MIC of each isolate. This is because of the prevalent use of reflective testing to verify MICs. Otherwise, this result might show the existence of colistin hetero-resistance, that was designated in *K. pneumoniae* and *A, baumannii*, improved both from patients treated with, and inexpert to colistin prior to isolation of the organism. (9,10)

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