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Original Research Article

Comparison of colistin susceptibility testing by VITEK 2 compact automated system and broth microdilution method for gram-negative isolates in a tertiary care hospital

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Abstract

Background: The increasing global concern of colistin resistance, especially among multidrug-resistant bacteria, highlights the urgent need for enhanced surveillance. This study compared the VITEK 2 compact automated system with in-house broth microdilution (BMD) for Gram-negative isolates, assessing agreement and errors.

Materials and Methods: In this retrospective comparative analytic study conducted from March 1, 2023, to August 2023, a total of 879 Gram-negative isolates, including *Enterobacterales*, *Acinetobacter baumannii complex*, and *Pseudomonas aeruginosa* (P.A), were identified, tested, and analyzed according to CLSI M-100 Ed-33 and EUCAST version 13.1, 2023 guidelines for colistin susceptibility testing using the VITEK 2 compact automated system with reference to the in-house BMD method in a microbiology laboratory at a tertiary care hospital.

Results: Out of 879 isolates, colistin resistance rates were 3.75% (BMD) and 4.32% (VITEK 2). VITEK 2 missed resistance in 2 isolates, per EUCAST guidelines. Comparing VITEK 2 with in-house BMD for colistin susceptibility in *Enterobacterales*: the overall essential agreement (EA) was 83.4%, and the categorical agreement (CA) was 99.4%. The very major error (VME) rate was 5.88%, and the minor error (ME) rate was 0.41%. For *Pseudomonas aeruginosa*, the overall EA was 71.5%, with a CA of 97%. The VME rate was 1%, and the ME rate was 2.68%. Regarding the *Acinetobacter baumannii complex*, EA was 76.53%, CA 100%, with no ME or VME as per the guidelines.

Conclusion: Discrepancies in colistin susceptibility testing were noted. BMD should confirm VITEK 2 results for both resistant and susceptible isolates.

Keywords: Broth microdilution; Colistin susceptibility testing.

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1. Introduction

Multidrug-resistant bacteria (MDR), especially Gram-negative ones, are a major global health threat, causing higher death and illness rates. Colistin, once a last-resort antibiotic, is now vital for treating these tough infections. However, resistance to colistin has become a serious issue due to the lack of alternative antibiotics.[1]

Polymyxins, such as Polymyxin B and colistin, are penta-cationic antibiotics that target Gram-negative bacteria by binding to the lipopolysaccharide (LPS) present on the bacterial cell wall.[2] However, resistance to these antibiotics has become increasingly prevalent, particularly among organisms responsible for nosocomial infections, such as *Enterobacterales*, as well as non-fermenters like *Acinetobacter baumannii (A. baumannii)* and *Pseudomonas aeruginosa (P. aeruginosa)*. This resistance is facilitated through various mechanisms, including efflux mechanisms, cationic modification of lipopolysaccharide, and impermeability. The genetic basis for resistance involves both plasmid and chromosomal-mediated mechanisms.[3]

Testing for colistin susceptibility presents significant challenges due to several factors, including poor diffusion in agar, the cationic properties of colistin, and heteroresistance

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in MDR organisms.[4] According to joint CLSI-EUCAST guidelines, the broth microdilution method (BMD) is the gold standard for testing the Minimum Inhibitory Concentration (MIC) for colistin. However, it is often deemed laborious and time-consuming.[4] Nevertheless, automated systems have emerged as the cornerstone of diagnostic microbiology labs in developing countries, facilitating the identification and antibiotic susceptibility testing of clinical isolates due to their ease of use and efficiency.[1]

This study primarily evaluates the performance of the VITEK 2 compact automated system in detecting the susceptibility of colistin, which is compared with the in-house broth microdilution (BMD) method for *Enterobacterales* and non-fermenters such as *Acinetobacter spp*. and *Pseudomonas spp*. The objective is to assess the degree of agreement between these methods and identify different types of errors in colistin susceptibility testing by the VITEK 2 compact automated system.

2. Materials and Methods

A retrospective comparative study was conducted at a tertiary care hospital from March 1, 2023, to August 31, 2023. We examined 879 non-duplicate Gram-negative bacteria (GNB) isolated from clinical samples such as blood, urine, pus, tracheal secretions, and sputum from hospitalized patients. We excluded inherently colistin-resistant bacteria like *Morganella* spp., *Proteus* spp., *Providencia* spp., *Serratia marcescens, and Burkholderia* spp. No informed consent was required, as the study did not involve human subjects. The study was approved by the Institutional Review Board (IRB).

The clinical samples underwent standard laboratory procedures in the Microbiology laboratory. As per the departmental policy, all culture-positive samples were subjected to colony identification by Gram stain, followed by analysis using the VITEK 2 compact automated system with Gram-negative (GN) and Gram-positive (GP) cards. Antibiotic susceptibility testing with the VITEK 2 compact automated system was performed using AST 405 and AST 406 cards (Both AST cards include colistin as one of the drugs being tested). Additionally, broth microdilution (BMD) was conducted to determine the colistin Minimum Inhibitory Concentration (MIC) against all selected GNB isolates in a 96-well polystyrene microtiter plate.[6-8]

Colistin sulphate powder was diluted with sterile distilled water to prepare a working stock solution of 64 µg/ml in microcentrifuge tubes, resulting in a final concentration of 16 µg/ml in the microtiter plate wells. Serial two-fold dilutions were then made from the working stock solution to obtain the desired concentrations for the colistin susceptibility test, ranging from 0.25 µg/ml to 16 µg/ml, following guidelines. Each well of the microtiter plate received 25 µl of the two-fold serially diluted colistin solutions and 50 µl of (CAMHB) cation-adjusted Mueller-Hinton broth, except for the growth control and media control wells.

Colonies from non-selective media, incubated overnight at 37°C aerobically, were dissolved in normal saline to achieve a 0.5 McFarland turbidity standard (1.5×108 CFU/ml*).* This was further diluted 1 in 75 to obtain the standardised inoculum *(2*×106 CFU/ml) in saline. From this, 25 µl of the inoculum was added to each well within 15 minutes of its preparation to achieve the final inoculum in each test well (5 × 104 CFU/ml). The inoculated microtiter plates were then incubated at 35 ± 2°C for 16–18 hours, after which the result was recorded visually and interpreted. (**Table 1**) Each row contained a growth control to verify the organism's viability and a negative control to check for broth media contamination. Quality control strains used during the experiments were *E. coli* ATCC 25922 (range: 0.25–2 µg/ml) and *P. aeruginosa* ATCC 27853 (range: 0.5–4 µg/ml).

Table 1: Colistin interpretative breakpoints according to CLSI M-100 Ed-33 guidelines and EUCAST version 13.1,2023 guidelines.[6,7]

Organism	CLSI 2023		EUCAST 2023	
	I	R	S	R
Enterobacterales	≤2	≥4	≤2	>2
Pseudomonas aeruginosa	≤2	≥4	≤4	>4
Acinetobacter baumannii complex	≤2	≥4	≤2	>2

S- Susceptible; I- intermediate; R- Resistant

3. Results

A total of 879 clinical isolates of Gram-negative bacteria were collected and tested for colistin susceptibility using both BMD and the VITEK 2 compact automated system. The majority of these isolates came from swab exudate samples (35.27%), followed by urine (20.93%), respiratory samples (17.63%), blood (12.06%), and sputum (6.37%). Fluid (1.02%) and pus (2.62%) samples were least frequently isolated.

Out of the 879 isolates, 56.88% were from *Enterobacterales*, including *E. coli* (28.10%), *Klebsiella pneumoniae* (25.71%), *Enterobacter cloacae complex* (2.16%), and *Citrobacter* (0.91%). The remaining 43.12% were non-fermenters, primarily *Pseudomonas aeruginosa* (22.75%) and *Acinetobacter baumannii complex* (20.36%). *E. coli* was the most common organism isolated from Urine samples (38.46%) and (Swab) Exudates (27.53%), followed by *K. pneumoniae* (Swab Exudates: 41.15%, Urine: 18.14%). Regarding patient location, the majority of isolates, 768 (87.37%) were from indoor patients, with 436(56.77%) from various wards and 332(43.23%) from the ICU. Outpatients accounted for 111(12.63%) isolates.

When analysing colistin interpretive results, the VITEK 2 compact automated system and in-house BMD were compared. The *Acinetobacter baumannii complex* demonstrated complete categorical agreement, reaching 100%. Following closely behind, the *Enterobacterales* displayed a high categorical agreement of 99.4%, while *Pseudomonas aeruginosa* showed a slightly lower but still substantial categorical agreement rate of 97%.

When comparing colistin susceptibility results for *Enterobacterales* from the VITEK 2 compact automated system versus the reference broth microdilution method, according to CLSI guidelines, the overall essential agreement was 83.4% (417 out of 500) with an overall essential disagreement rate of 16.6% (83 out of 500). Minor errors were exceptionally low at 0.6% (1 out of 625). According to EUCAST 2023 guidelines, very major errors were observed in 5.88% (1 out of 17) of cases and major errors in 0.41%(2 out of 483) of cases. **Table 2**)

The overall essential agreement for *Pseudomonas aeruginosa* was 71.5% (143 out of 200), while the essential disagreement was 28.5% (57 out of 200). There was a minor error rate of 21% (42 out of 200) according to CLSI guidelines. In addition, there was a very major error of 7.14% (1 out of 14) and a major error of 2.68% (5 out of 186) based on EUCAST 2023 guidelines. (**Table 2**)

For *Acinetobacter baumannii complex*, the overall essential agreement was 76.53% (137 out of 179), with an overall essential disagreement of 23.46% (42 out of 179). There were no minor errors observed as per CLSI guidelines, and no major or very major errors were found according to EUCAST 2023 guidelines. (**Table 2**)

The VITEK 2 compact automated system shows a sensitivity of 99.17% when compared to BMD, along with a specificity of 93.94%. In the current study, the positive predictive value (PPV) is 99.76%, while the negative predictive value (NPV) is recorded at 81.57% for all isolates. (**Table 3**)

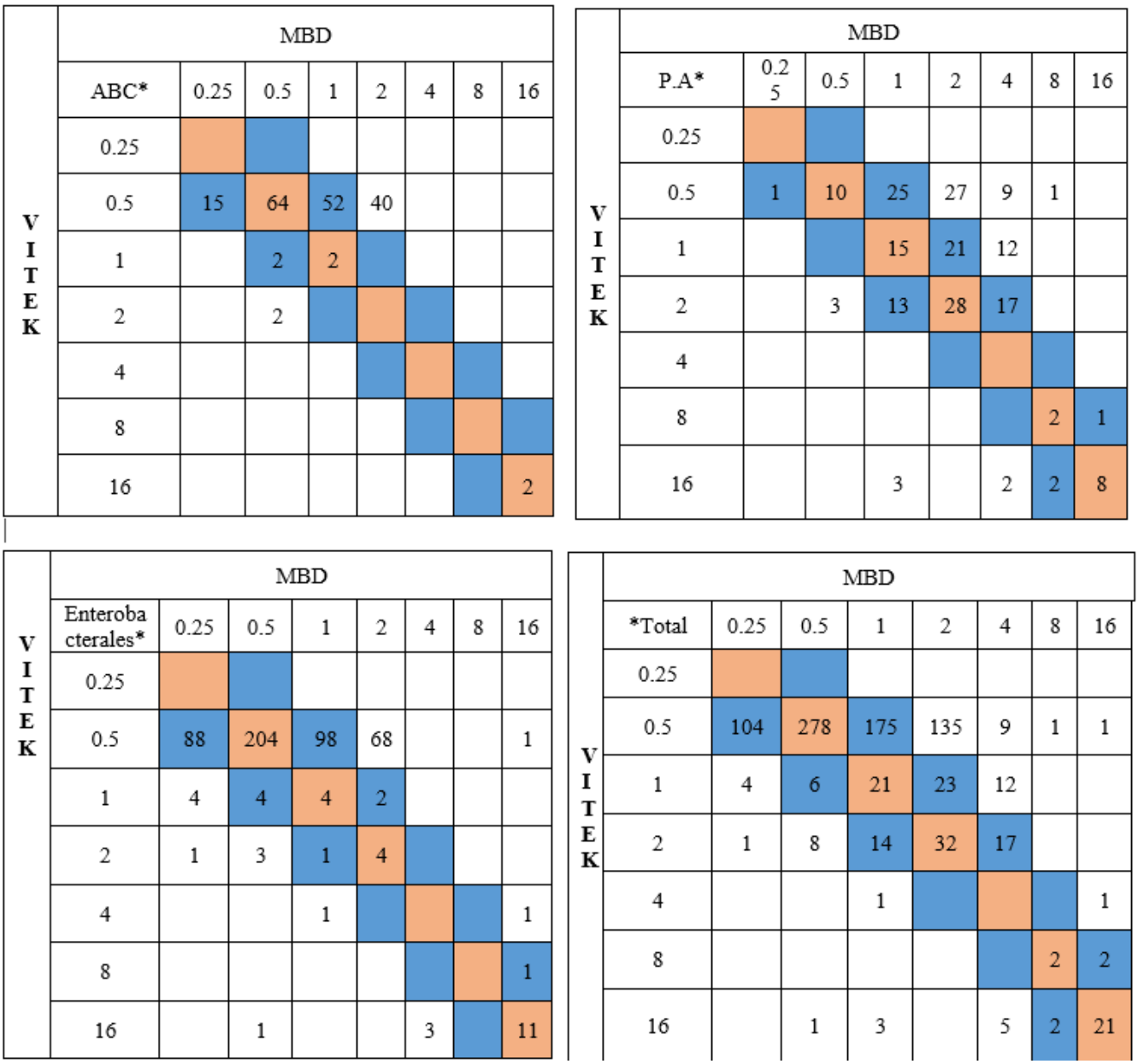
Table 2: Agreement of VITEK 2 compact automated system method with the reference in-house BMD method for individual organisms

Organism	**Categorical agreement n (%)**	**Categorical disagreement**		**Essential agreement**	
		Major error n(%)	Very major error n(%)	Agreed n(%)	Disagreed n (%)
Enterobacterales (N=500)	497 (99.4%)	2 (0.41%)	1 (5.88%)	417 (83.4%)	83 (16.6%)
Pseudomonas aeruginosa (N=200)	194 (97%)	5 (2.68%)	1 (7.14%)	143 (71.5%)	57 (28.5%)
Acinetobacter baumannii complex (N=179)	179 (100%)	0	0	137 (76.53%)	42 (23.46%)

N: Number of isolates

Table 3: Performance characteristics of Broth Microdilution and VITEK 2 compact automated system methods

Organisms	**VITEK®2**	**BMD**		**Sensitivity%**	**Specificity%**	**PPV%**	**NPV%**
		Sensitive	**Resistant**				
Enterobacterale s (n = 500)							
E. coli(n=247)	S	247	0	100	NA	100	NA
	R	0	0				
K. pneumoniae(n=226)	S	208	1	99.05	93.75	99.52	88.23
	R	2	15				
Enterobacter cloacae complex (n=19)	S	18	0	100	100	100	100
	R	0	1				
Citrobacter(n=8)	S	8	0	100	NA	100	NA
	R	0	0				
Non-fermenters (n = 379)							
P. aeruginosa(n=200)	S	177	0	97.31	92.86	99.45	72.22
	R	0	2				
A. baumannii(n=179)	S	181	1	100	100	100	100
	R	5	13				



VITEK \ MBD	ABC* 0.25	0.5	1	2	4	8	16
0.25							
0.5	15	64	52	40			
1		2	2				
2		2					
4							
8							
16							2

VITEK \ MBD	P.A* 0.25	0.5	1	2	4	8	16
0.25							
0.5	1	10	25	27	9	1	
1			15	21	12		
2		3	13	28	17		
4							
8						2	1
16			3		2	2	8

VITEK \ MBD	Enterobacterales* 0.25	0.5	1	2	4	8	16
0.25							
0.5	88	204	98	68			1
1	4	4	4	2			
2	1	3	1	4			
4			1				1
8							1
16		1			3		11

VITEK \ MBD	*Total 0.25	0.5	1	2	4	8	16
0.25							
0.5	104	278	175	135	9	1	1
1	4	6	21	23	12		
2	1	8	14	32	17		
4			1				1
8						2	2
16		1	3		5	2	21

Shaded in orange = number of isolates with identical MIC Shaded in blue = MICs within the essential agreement (within one-fold dilution) * ABC- *Acinetobacter baumannii complex** P.A- *Pseudomonas aeruginosa** *Enterobacterales**-Total of all *Enterobacterales* *Total-ABC+P.A+ *Enterobacterales*

Figure 1: Correlation of VITEK 2compact automated system method with reference inhouse BMD method.

4. Discussion

According to the study findings, *E. coli* caused the most infections (28.10%), followed by *Klebsiella pneumoniae* (25.71%), *Pseudomonas aeruginosa* (22.75%), and *Acinetobacter baumannii complex* (20.36%). Other organisms like *Enterobacter cloacae complex* and *Citrobacter* were responsible for fewer infections. Similar findings were reported in studies by Ananda et al and Gupta et al.[2,9]

In this study, 33 isolates (3.75%) were resistant to colistin using the BMD method, and 38 isolates (4.32%) were resistant using the VITEK 2 compact automated system. The VITEK 2 compact automated system missed detecting resistance in 2 isolates, similar to a study by Gupta et al.[9]

According to ISO 20776-2 and FDA guidelines, each test method should ideally achieve a ≥90% essential and categorical agreement level. Very major errors (VME) should not exceed 1.32%, and major errors (ME) should be ≤3%.[2,10]

The highest overall essential agreement was 83.4%, seen in *Enterobacterales*. However, all isolate groups showed a CA value of ≥90%, meaning the VITEK 2 compact automated system accurately detected colistin sensitivity compared to the BMD method. Similarly, in the present study, an acceptable very major error (VME) rate was <3%

in *Pseudomonas aeruginosa* and *Enterobacterales* groups, although major error (ME) rates were within the acceptable range for these organisms. VME and ME were not detected in *Acinetobacter baumannii complex* isolates. The highest VME rate was in *Pseudomonas aeruginosa* at 7.14%. Similar findings were reported in studies conducted by Ananda et al., Butta et al., and Zhu et al.[2,11,12] (**Table 2**).

In a study by Ananda et al., the essential agreement (EA) value was 68.5%, which is lower than the acceptable range. However, the categorical agreement (CA) value was 99.79%, falling within the acceptable range. Very major error (VME) rates varied widely between isolates, ranging from 47% to 100%, while major error (ME) reached up to 20%, which exceeded the standard guidelines. The highest VME was observed in *E. coli* at 100%.[2,13]

In another study by Butta et al. and Zhang *et al. Enterobacterales* isolates had ME within the acceptable range of <3%, but their CA, EA, and VME values were out of range. *Pseudomonas aeruginosa* isolates showed agreement in all aspects, with no VME detected. Additionally, no ME was observed in *Acinetobacter baumannii complex* isolates, with a CA of over 90%.[11,14]

In this study, the VITEK 2 compact automated system showed over 90% sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for both overall and individual tested organisms, except for *Pseudomonas aeruginosa*, where the NPV was below 90%. (**Table 3**) Ananda et al., reported similar findings where the VITEK 2 compact automated system demonstrated over 90% sensitivity, PPV, and NPV, but less than 90% specificity for all tested organisms.[2] In another study by Bidyutprava Rout et al., the VITEK 2 compact automated system showed over 90% specificity and NPV, but less than 90% sensitivity in PPV for all tested organisms.[15]

In our study, we found that *Acinetobacter baumannii complex*, *Pseudomonas aeruginosa*, and *Enterobacterales* isolates exhibited the highest essential agreement (ED) at a minimum inhibitory concentration (MIC) of 2µg/ml in BMD. Interestingly, all the isolates with discrepancies had MIC values of 0.5µg/ml in the VITEK 2 compact automated system. This indicates that the VITEK 2 compact automated system tends to report lower MIC ranges than the gold standard test, potentially suggesting increased susceptibility to colistin in these isolates. Therefore, it's advisable to verify resistant and susceptible colistin isolate results using the VITEK 2 compact automated system alongside an in-house BMD method. (**Figure 1**)

All significant clinical isolates responsible for severe and nosocomial infections, irrespective of their specific location or any particular phenotypic resistance mechanism, were included in this study. However, the VITEK 2 compact automated system failed to produce results within an acceptable range according to ISO 20776–2 and FDA guidelines. The very major error (VME) rate exceeded 3% for both *Enterobacterales* and Pseudomonas aeruginosa isolates, while the overall essential agreement (EA) fell below 90% for all tested organisms. Notably, Pseudomonas aeruginosa isolates exhibited the highest VME rate. 7.14% and poor EA. These discrepancies in colistin sensitivity reported by the VITEK 2 compact automated system could result in incorrect patient treatment management, potentially leading to increased morbidity and mortality.

5. Conclusion

This study demonstrates that while the VITEK 2 compact automated system meets acceptable categorical agreement (CA ≥ 90%) for colistin susceptibility testing, it fails to achieve essential agreement (EA ≥ 90%) and shows unacceptably high very major error (VME) rates, particularly in *Pseudomonas aeruginosa* and *Enterobacterales*. Notably, all discrepant isolates had MIC values of 0.5µg/ml in the VITEK 2 compact automated system, whereas the BMD method reported higher MICs, indicating a tendency of the VITEK 2 compact automated system to underestimate MICs and potentially overcall susceptibility. These findings highlight the risk of false susceptibility results, which can lead to inappropriate treatment, especially in cases of multidrug-resistant infections. Therefore, it is strongly recommended that both susceptible and resistant results obtained via the VITEK 2 compact automated system should be confirmed using the BMD method. Laboratories should exercise caution when using the VITEK 2 compact automated system for colistin susceptibility testing and consider adopting or maintaining BMD-based protocols to ensure accurate detection of resistance and avoid potential clinical mismanagement.

6. Ethical Statement

Institutional IRB no: NHLIRB/2023/AUGUST/3/NO.7 Date-4/8/2023.

7. Source of Funding

None.

8. Conflict of Interest

None.

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