Indian Journal of Microbiology Research 2024;11(4):243–247

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Indian Journal of Microbiology Research

Journal homepage: https://www.ijmronline.org/

Original Research Article

Comparative evaluation of broth microdilution, E-strip and Vitek-2 for colistin susceptibility among carbapenem resistant *Acinetobacter* Spp and *Klebsiella* Spp

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ARTICLE INFO

Article history:
Received 20-04-2024
Accepted 14-10-2024
Available online 09-12-2024

Keywords:
Broth microdilution
Carbapenem
Colistin
E- test
Vitek 2
Acinetobacter
Klebsiella

ABSTRACT

Background: Gram-negative bacterial infections that are resistant to carbapenems are a serious clinical problem. The only effective drugs against them are still tigecycline and colistin. In fact, polymyxin E, also known as colistin, has been regarded as a "last resort" antibiotic. But in recent years, colistin resistance has increased globally, significantly reducing treatment options and highlighting the significance of accurate colistin testing methods to support appropriate therapeutic decision-making.

Materials and Methods: The primary goal of the study is to compare the effectiveness of E-test and Vitek 2 for colistin sensitivity testing to the conventional microbroth dilution method using 120 clinical isolates of *Klebsiella pnuemoniae* and *Acinetobacter baumannii* that are resistant to carbapenem over the course of a year, isolated from January to December 2019.

Results: In comparison to Vitek-2, the results indicated that brothmicro dilution and E-test produced the narrowest range of colistin MICs. After comparing the E-test's errors and overall agreement with BMD, 100% of the Essential Agreement, 94.27% of the Categorical Agreement, and 5.72% of the Very Major Errors with no Major Errors were found. Comparing Vitek-2 with BMD, similar results were found: 1.66% MEs, 98.32% CAs, 80.99% EAs, and no VMEs.

Conclusion: As a result, it was determined that, in contrast to Vitek-2, MBD and E-test had distinct MICs, making them appropriate for determining colistin MIC. It will take more research on automated systems to standardize colistin susceptibility testing procedures, particularly for *K. pnuemoniae* and *A. baumannii*.

1. Introduction

Gram-negative bacterial infections that are resistant to carbapenems pose a serious clinical risk. Recently, there has been an increase in the frequency of infections caused by carbapenem-resistant organisms, particularly in *K. pnuemoniae* and *A. baumannii*.[1]

The carbapenem class has long been regarded as the best medication class for treating serious *A. baumannii* infections. The carbapenem class's dependability has been called into question due to the fast rising prevalence of carbapenem-resistant *A. baumannii* (CR-Ab) isolates in various regions of the world.[2,3]

Two significant multidrug-resistant and carbapenem-resistant organisms in healthcare are K. pneumoniae and *A. baumannii*. Plasmid-coded carbapenemase, which has surfaced worldwide and raised serious concerns, mediates resistance to carbapenem.[2,4] Since the presence of carbapenemases might start actions to stop the lateral development of resistance and possible outbreaks, their activity identification has a significant influence on the management of hospital infections.[4,5]

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https://doi.org/10.18231/j.ijmr.2024.043

The usage of outdated and neglected antibiotics like polymyxin (polymyxin B) and colistin has increased due to the rise of carbapenem-resistant and multidrug-resistant infections.[5]

The only effective antibiotics for treating (MDR) pathogens are tigecycline and colistin. Tigecycline is advised for skin infections, community-acquired pneumonia, and intra-abdominal infections.[2]

Polymyxin has been a commonly utilized antibiotic alternative in recent decades to combat organisms that are resistant to carbapenem. As a matter of fact, polymyxin E, also known as colistin, was often the only antibiotic that could be used to treat MDR *K. pneumoniae* and *A. baumannii* infections when it came to minimum inhibitory concentrations (MICs and serum levels.[6,7]

Global reports of rising colistin resistance in recent years have further restricted treatment options.[8] For appropriate treatment decision-making in standard clinical laboratories, rapid and efficient colistin susceptibility testing (ST) is necessary. In order to determine colistin susceptibility, clinical laboratory standard institute (CLSI), Pittsburg, USA advised using microbroth dilution (MBD) until 2019. However, according to the 2020 CLSI guidelines, the colistin disk elution method and the micro broth dilution method should be used to determine colistin susceptibility for the group of Enterobacteriaceae.[9] The effectiveness of the colistin sensitivity testing method has only been partially studied, with inconsistent results, and even the most accurate methods are difficult to use. Disk diffusion (DD) is frequently employed in other labs, but due to its greater error rates, it is not thought to be a accurate method for detecting colistin resistance.[10,11]

In clinical microbiology laboratory diagnostics, the use of the MBD reference method for colistin sensitivity testing might not be feasible (reference). For the purpose of determining the MIC, the Vitek 2 and the E-test were utilized as alternatives.[12–14] In order to compare the E-test and Vitek 2 for colistin sensitivity testing to the CLSI-recommended MBD method, this study was conducted.

2. Materials and Methods

Clinical samples, including sputum, Endotracheal aspirate, and other bodily fluids such as blood, pus and urine were processed on blood agar and Mac-conkey agar in a tertiary care hospital at Mysuru, Karnataka, India for this study. Using GN ID cards with the N281 and N280 drug panels, respectively, carbapenem-resistant *A. baumannii* and *K. pnuemoniae* were identified by VITEK-2 (bioMérieux, Mysore, India) in accordance with manufacturer's instructions.[15]

2.1. Colistin-resistance detection methods

1. The Colistin Ezy MICTM Strip (0.016-256 mcg/ml) (LOT No.: 0000375203 HiMedia Laboratories Mysuru, Karnataka, India) was used for the E-strip method in accordance with manufacturer's instructions, along with the proper controls (ATCC *E. coli* 25922 and *Ps. aeruginosa* 27853).[15]
2. Using the MIKROLATEST kit (LOT: 1710152 from Erba Lachema s.r.o., Karásek 2219/1d, 621 00 Brno, CZ) and CLSI 2019 with the proper controls (ATCC *Ps. aeruginosa* 27853 and ATCC *E. coli* 25922), the MBD method was carried out.[6] Analyses and comparisons were made between the VITEK-2, E-strip, and broth microdilution results.

3. Results

Of total 120 carbapenem resistant isolates, 57(47.5%) were CR *A. baumannii* and 63 (52.5%) were CR *K. pneumoniae*. Comparing E-test to BMD**,** narrowest colistin MIC of 1μg/ml were observed in both E-strip (0.5, 1, 1.5, and 2) and BMD (0.5, 1, 2 and 4) for majority of the samples. i.e. 63.15% & 82.45% from BMD and E-test respectively. Comparing Vitek-2 to BMD**,** BMD showed narrowest range (0.5, 1, 2 and 4) than Vitek-2 (0.5, 16). Majority of samples showed MIC 1μg/ml by BMD and 0.5μg/ml by vitek-2 (Table 1). Hence determination of different ranges of colistin MIC by Vitek-2 appears to create difficulty. The P value detected by statistical analysis (SPSS 16.0V) is 0.001 for both VITEK-2 and E-strip in comparison with BMD.

3.1. Calculation of essential agreement (EA), categorical agreement (CA), Very major errors (VME) and major errors (ME)

EA was defined as MICs that were within ±1 log2 dilution. MIC agreement was evaluated within 0.5–16 μg/ml, as the MIC ranges for Etest (0.5–2 μg/ml) and Vitek 2 (0.5–16 μg/ml) differed from BMD (0.5-4 μg/ml). Bacteria with colistin MIC ≥2 μg / ml were considered as resistant, CA was calculated using this breakpoint. Result of BMD was taken as gold standard to compare other two methods. A false-intermediate result was considered as VME and false-resistance result as ME**.**

In 57 CR *A. baumannii*, EA between Etest and BMD is 100%, CA 98.24% with 3.5% VME and no ME (Figure 1 and Table 2). EA between the Vitek-2 and BMD is 84.21%, CA 98.24% with no VME and 1.75% ME (Figure 2 and Table 2).

Vitek-2 produced 84.21% EA overall and 21% showed MICs similar to BMD 2 and 3 log2 dilutions higher than BMD was for 3.5%, and 1.75% respectively. 63.15% and 10.52% showed MICs that were 1 and 2 log2 dilutions lower than BMD**.** E-test developed 100% EA overall and 70.1% showed MICs similar to BMD. 1 log2 dilutions more than

Table 1: Comparitive evaluation of different MIC values Vitek-2 and E-strip method with reference broth microdilution method in *A. baumannii* (n=57) and *K. pnuemoniae* (n=63)

MIC Value	BMD (*A. baumannii*)	BMD (*K . pnuemoniae*)	Vitek-2 (*A. baumannii*)	Vitek-2 (*K. pnuemoniae*)	E-strip (*A. baumannii*)	E-strip (*K. pnuemoniae*)
0.5	12 (21.05%)	3 (4.76%)	54 (94.73%)	57 (90.47%)	3 (5.26%)	1 (1.58%)
1	36 (63.15%)	46 (73.015%)	0 (0%)	0 (0%)	47 (82.45%)	37 (58.73%)
1.5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (7.01%)	19 (30.15%)
2	7 (12.28%)	9(14.28%)	0 (0%)	0 (0%)	3 (5.26%)	6 (9.52%)
4	2 (3.50%)	5 (7.93%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
16	0 (0%)	0 (0%)	3 (5.26%)	6 (9.52%)	0 (0%)	0 (0%)

Table 2: Calculation of EA, CA, VMEs and MEs of colistin MICs in *A. baumannii* amongst the E-test, Vitek-2 with BMD

		E-test				Vitek-2			
Organism Tested	No. Tested	EA	CA	VME	ME	EA	CA	VME	ME
A. baumannii	57	57 (100%)	55 (96.49%)	2 (3.5%)	0	48 (84.21%)	56 (98.24%)	0	1 (1.75%)

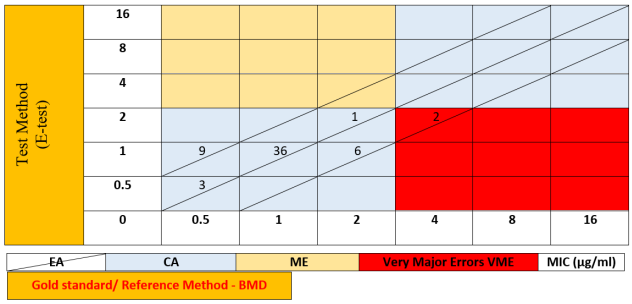

Figure 1: Scatter-gram in *A. baumannii* (n=57) with E-test versus MBD. Diagonal line indicates EA

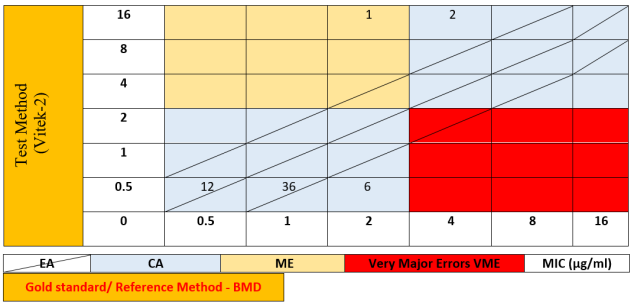

Figure 2: Scatter-gram in *A. baumannii* (n=57) with Vitek-2 versus MBD. Diagonal line indicates Essential agreement

BMD is 15.7% and 14% 1 log2 dilution lower than BMD (Table 3).

In 63 CR *K. pnuemoniae*, Comparing E-test to BMD, narrowest colistin MIC of 1μg/ml were observed in both E-strip (0.5, 1, 1.5, and 2) and BMD (0.5, 1, 2 and 4) for majority of the samples. i.e.73.15% & 58.73% from BMD and E-test respectively. Comparing Vitek-2 to BMD, narrowest CL MICs is observed only in BMD (0.5, 1, 2 and 4) compared to Vitek-2 (0.5 and 16). 73.15% showed 1μg/ml by BMD and 90.47% showed 0.5μg/ml by vitek-2 (Table 1). Hence determination of different ranges of colistin MIC by Vitek-2 appears difficult. P-value is 0.001 for both Vitek-2 & E-test with BMD.

EA between Etest and BMD is 100%, CA 92.06% with 7.94% VMEs and no MEs (Figure 3 and Table 4). EA between Vitek-2 & BMD is 77.77%, CA 98.41% with no VMEs and 1.58% ME (Figure 4and Table 4).

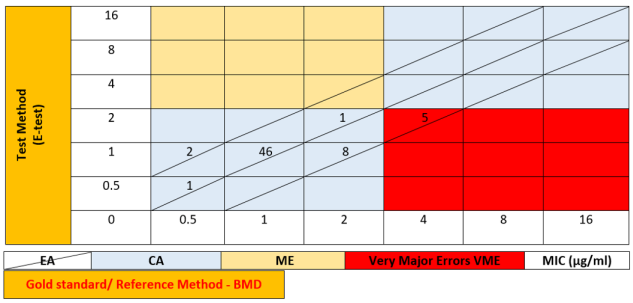

Figure 3: Scatter-gram in *K. pnuemoniae* (n=63) with colistin MICs detected by E-test versus MBD as reference. Break point for susceptibility (≤2μg/ml). The diagonal line indicates Essential agreement

Vitek-2 generated 77.77% EA overall. Only 4.76% showed identical MICs to BMD. 2 and 3 $\log_2$ dilutions higher than BMD is 7.93%, and 1.58%. 73.01% and 12.69% displayed 1 and 2 $\log_2$ dilutions lower than BMD. E-test generated 100% EA overall and 76.19% showed identical MICs to BMD. 3.17% displayed 1 $\log_2$ dilutions higher and 20.63% displayed 1 $\log_2$ dilution lower than BMD (Table 5).

4. Discussion

This study was carried out to evaluate the performances of three colistin susceptibility testing methods (Vitek-2, E-test and MBD method) against carbapenem resistant *A.*

Table 3: EA in *A. baumannii* and $\log_2$ dilutions difference of MICs by Vitek-2 and E-test compared to BMD method

Method compared	-3	-2	-1	0	+1	+2	+3
Vitek-2	0	6 (10.52%)	36 (63.15%)	12 (21%)	0	2 (3.5%)	1 (1.75%)
E-test	0	0	8(14%)	40 (70.17%)	9 (15.7%)	0	0

Table 4: Calculation of EA, CA, VME and ME of CL MICs in *K. pnuemoniae*

		E-test				Vitek-2			
Organism	Total	EA	CA	VME	ME	EA	CA	VME	ME
K. pnuemoniae	63	63 (100%)	58 (92.06%)	5 (7.94%)	0	49 (77.77%)	62 (98.41%)	0	1 (1.58%)

Table 5: EA in *K. pnuemoniae* and $\log_2$ dilutions difference MICs of Vitek-2 & E-test compared to BMD

Method compared	-3	-2	-1	0	+1	+2	+3
Vitek-2	0	8 (12.69%)	46 (73.01%)	3 (4.76%)	0	5 (7.93%)	1 (1.58%)
E-test	0	0	13 (20.63%)	48 (76.19%)	2 (3.17%)	0	0

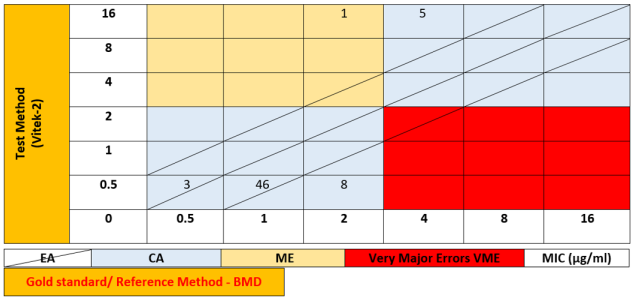

Figure 4: Scatter-gram in *K. pnuemoniae* isolates (n=63) with colistin MICs detected by Vitek-2 versus MBD as reference. Break point for susceptibility (≤2μg/ml). Diagonal line indicates Essential agreement

baumannii and *K. pnuemniae* clinical isolates. Our study included 120 clinical isolates collected over a period of 1 year from Jan-2019 to Dec-2019, of which 57 (47.5%) were carbapenem resistant *A. baumannii* and 63 (52.5%) were carbapenem resistant *K. pnuemoniae*. In our study, by comparing the E-test and Vitek-2 method with the Standard Reference BMD method, it was observed that BMD and E-test method generated narrowest range of colistin MICs (0.5 to 4μg/ml) when compared to Vitek-2 method which generated only two values for colistin MICs i.e. 0.5 and 16 μg/ml. A similar study conducted by Sueng Yeob Lee et al showed similar results i.e. narrowest range of colistin MICs distribution in broth microdilution (1μg/ml to 4μg/ml) and E-test (0.016 to 4μg/ml) when compared to vitek-2 method (0.5 and 16μg/ml) remarking that from both the study, Broth Microdilution and E-test methods were suitable for colistin suscetibility testing.[4,7]

In our study, of the 120 carbapenem resistant *A. baumannii* (57) and *K. pnuemoniae* (63), by comparing the Errors and total agreement of E-test with the Standard Reference BMD method, 100% of EA, 94.27% of CA, 5.72% VME and no ME were observed. But in the study conducted by T. Y Tan et al showed unlike results i.e. 75% of EA, 86.6% of CA, 4.7% VME and 8.7% ME remarking significant differences in both the study, concluding that more number of very major errors were seen in both the studies and no major error in seen in our study.[8,15]

Similarly by comparing the Errors and total agreement of Vitek-2 with the Standard Reference Broth microdilution method, our study showed 80.99% of Essential agreement, 98.32% of categorical agreement, no very major errors and 1.66% of major errors were observed. But in the study conducted by T. Y Tan et al[8] showed unlike results i.e. 75% of Essential agreement, 82% of categorical agreement, with 57.4% very major errors and no major errors remarking significant differences in both the study, concluding that more number of errors were seen in their study.

5. Conclusion

In contrast to Vitek-2, which displayed only two results for colistin susceptibility of *Acinetobacter spp.* and *K.pnuemoniae* the MBD and E-test procedures revealed distinct MICs for colistin, making them appropriate for determining colistin MIC stating that more research on automated systems is necessary. Nonetheless, the recently suggested Disk Elution Method (DEM) by CLSI, which was excluded from the current investigation, might be an additional easier and more successful method for determining colistin susceptibility.

6. Abbreviations

BMD: Broth Micro Dilution; MIC: Minimum Inhibitory Concentration; VME:- Very Major Error; ME: Major Error; EA: Essential Agreement; CA: Categorical Agreement; Cr-

Ab: Carbapenem resistant Acinetobacter baumannii

7. Research Quality and Ethics Statement

The authors of this manuscript declare that this scientific work complies with reporting quality, formatting and reproducibility guidelines set forth by the EQUATOR Network. The authors also attest that this study was determined to require the Institutional Ethics Committee review, and the corresponding approval number is JSSMCPG|227|2018-2019|Dated 02-02-2019. The authors hereby declare that this study has not been registered in any other journal.

8. Source of Funding

None.

9. Conflict of Interest

None to declare.

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Cite this article: Basavaraju M, Rao MR, Shankaregowda R, Mahale RP, Shivappa SG, Chitharagi VB. Comparative evaluation of broth microdilution, E-strip and Vitek-2 for colistin susceptibility among carbapenem resistant *Acinetobacter* Spp and *Klebsiella* Spp. *Indian J Microbiol Res* 2024;11(4):243-247.