

## Comparison of Colistin Susceptibility Testing by Vitek 2 Compact and Broth Microdilution Method for Carbapenem Resistant Isolates in a Tertiary Diagnostic Centre

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### ABSTRACT

A study was undertaken to compare colistin susceptibility using BMD and Vitek in carbapenem resistant gram negative isolates to evaluate the discrepancies and further course of action.

**Conclusion:** The broth micro dilution (BMD) technique is reliable and is easy to use method for determining the MIC of Colistin. The results correlated with Vitek system except for 2 isolates which showed very major errors which indicates that in case of resistance to Colistin by Vitek, broth dilution method must be used for correlation and to recheck the result. Also in case of Vitek system showing susceptibility to Colistin, we can safely report those isolates without doing micro broth dilution as we did not encounter any isolates which gave susceptible on Vitek and resistant on micro broth dilution method.

**Keywords:** Colistin, Vitek, Broth microdilution, MIC

### INTRODUCTION

Colistin also known as polymyxin E is an antibiotic produced by certain strains of the bacteria *Penicillium polymyxa*. Colistin is a mixture of the cyclic polypeptides colistin A and B and belongs to the class of polypeptide antibiotics known as polymyxins. Colistin is effective against most Gram-negative bacilli.

Colistin is a decades-old drug that fell out of favor in human medicine due to its kidney toxicity. It remains one of the last-resort antibiotics for multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter* [1]. NDM-1 metallo- $\beta$ -lactamase multidrug-resistant Enterobacteriaceae have also shown susceptibility to colistin [2].

Colistin has been effective in treating infections caused by *Pseudomonas*, *Escherichia* and *Klebsiella* species. Colistin is an effective antibiotic for treatment of most multidrug-resistant Gram-negative bacteria. It is used currently as a last-line drug for infections due to severe Gram-negative bacteria followed by an increase in resistance among Gram-negative bacteria.

Colistin resistance is considered a serious problem, due to a lack of alternative antibiotics. Some bacteria including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, Enterobacteriaceae members, such as *Escherichia coli* and *Klebsiella* spp. have an acquired resistance against colistin.

Colistin is increasingly needed for the treatment of infections caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates [3]. The accurate antimicrobial susceptibility testing (AST) of colistin is of obvious importance; however, considerable discrepancies have been reported between the available assays. To address this issue, EUCAST and CLSI recently formed a Polymyxin Breakpoints Working Group for colistin susceptibility testing [4], which recommended that broth micro dilution (BMD), is the most valid method for colistin AST. Among the diffusion methods, disc diffusion is unacceptable due to the large colistin molecule, while several studies in the literature have reported considerable discrepancies of the MICs produced by gradient tests [5]. The joint EUCAST/CLSI working group recently confirmed the problems that both of the available colistin gradient tests (manufactured by bioMérieux and Liofilchem) exhibit [6]. Colistin has been traditionally reported by all automated

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systems like VITEK, Phoenix since many years. CLSI guidelines 2018 issued a correction as follows - The only approved MIC method for testing is broth micro dilution method. Disc diffusion and gradient diffusion methods should not be performed. Biomerieux and BD have both issued a product correction notice on the same eventually in 2018.

**STUDY**

The broad objective of this study is to explore the concept of mental health healing among pastors and possibilities of collaboration with mental health professionals in Mzuzu.

Colistin susceptibility is done in our lab using MICROLATEST<sup>R</sup> marketed by Transasia in India. It is a broth micro dilution test which is CE=IVD approved for testing for Colistin. The cut offs provide are 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mcg/ml.

Breakpoints for Colistin to test *Pseudomonas* spp. and *Acinetobacter* spp. are as follows as per CLSI 2018. Resistant: >=4 mcg/ml, Susceptible: <=2 mcg/ml.

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Breakpoints for Colistin to test Enterobacteriaceae are as follows as per EUCAST 2019. Resistant: >=2 mcg/ml, Susceptible: <=2 mcg/ml.

We have followed EUCAST for Enterobacteriaceae and CLSI for *Pseudomonas* spp. and *Acinetobacter* spp. Recommendations for MIC determination of colistin (polymyxin E).

As recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group published at <http://www.eucast.org> on 22 March, 2016 [4,7].

Colistin (polymyxin E) MIC determination is associated by several methodological issues. The issues have been

extensively investigated by the CLSI-EUCAST joint Polymyxin Breakpoints Working Group and the following method for determination of colistin MIC was agreed:

1. Reference testing of Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. is by the ISO-standard broth micro dilution method (20776-1).

Note:

- a. Cation-adjusted Mueller-Hinton Broth is used.
  - b. No additives may be included in any part of the testing process (in particular, no polysorbate-80 or other surfactants).
  - c. Trays must be made of plain polystyrene and not treated in any way before use.
  - d. Sulphate salts of polymyxins must be used (the methane sulfonate derivative of colistin must not be used - it is an inactive pro-drug that breaks down slowly in solution).
2. Susceptibility testing by other methods, including agar dilution, disk diffusion and gradient diffusion, cannot be recommended until historical data have been reviewed or new study data have been generated. Work on these methods is ongoing.

**RESULTS**

A total of 90 isolates over the 2 months were studied (July-August 2019). All the isolates were carbapenem resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae.

The carbapenem resistant isolate distribution was as follows:

1. Carbapenem resistant Enterobacteriaceae are a majority of the isolates which comprises of 71.11% of all the isolates.

Isolates	No. of isolates	% of isolates
Enterobacteriaceae	64	71.11
<i>Klebsiella pneumoniae</i>	40	44.44
<i>E. coli</i>	21	23.33
<i>Enterobacter aerogenes</i>	03	3.33
Non-fermenters	26	28.89
<i>Pseudomonas aeruginosa</i>	19	21.11
<i>Acinetobacter baumannii</i>	07	7.77
<b>Total</b>	<b>90</b>	<b>100</b>

2. The sample distribution for carbapenem resistant gram negative isolates is as follows:

Sample types	No. of isolates	% of isolates
Urine	61	67.77
Pus	10	11.11
Sputum	09	10
E.T. secretions	06	6.66
Blood	03	3.33
Bile	01	1.11
Total	90	100

3. Urine forms the bulk of samples with carbapenem resistant gram negative isolates (67.77%). The organism distribution sample wise is as follows:

Sample type	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>
Urine	17	26	02	12	02
Pus	02	02	01	03	02
Sputum	01	07	00	03	00
ET secretions	00	03	00	01	02
Blood	00	02	00	00	01
Bile	01	00	00	00	00
Total	21	40	03	19	07

4. *Klebsiella* causing UTI is the predominant isolate-sample wise followed by *E. coli* and *Pseudomonas* in urine. MIC distribution in gram negatives by BMD is as follows:

Organisms	No. of isolates	MIC ≤0.5 mcg/ml		MIC 1 mcg/ml		MIC: 2 mcg/ml		MIC 4 mcg/ml		MIC >16 mcg/ml	
		VTK	BMD	VTK	BMD	VTK	BMD	VTK	BMD	VTK	BMD
<i>E. coli</i>	21	21	20	-	-	-	01				
<i>Klebsiella pneumoniae</i>	40	39	40	-	-	-	-	-	-	01	-
<i>Enterobacter aerogenes</i>	03	03	03	-	-	-	-	-	-	-	-

5. 1 out of 21 *E. coli* isolates showed discrepancy, and 1 out of 40 *Klebsiella pneumoniae* isolates showed discrepancy. 3 *Enterobacter* isolates showed no discrepancy.

Organisms	No. of isolates	MIC ≤0.5 mcg/ml		MIC 0.5-1 mcg/ml		MIC 1-2 mcg/ml		MIC 2-4 mcg/ml		MIC >4 mcg/ml	
		VTK	BMD	VTK	BMD	VTK	BMD	VTK	BMD	VTK	BMD
<i>Pseudomonas aeruginosa</i>	19	18	19	-	-	-	-	-	-	01	-
<i>Acinetobacter baumannii</i>	07	07	07	-	-	-	-	-	-	-	-

6. 1 *Pseudomonas* isolate showed MIC discrepancy resulting in major error in interpretation. 7 isolates showed minor difference in MIC values.

Details of the discrepancy:

Isolates	Mic by Vitek	Mic by BMD	Type of errors
Klebsiella - Urine	≥16.0	0.25	Very major error
<i>E. coli</i> - Sputum	≤0.5	2.0	Minor
<i>E. coli</i> - Pus	≤0.5	1.0	Minor
<i>E. coli</i> - urine	≤0.5	1.0	Minor
<i>Pseudomonas</i> - ET secretions	≤0.5	1.0	Minor
<i>Pseudomonas</i> - Urine	≤0.5	2.0	Minor
<i>Pseudomonas</i> - Urine	≤0.5	1.0	Minor
<i>Pseudomonas</i> - Urine	≤0.5	1.0	Minor
<i>Pseudomonas</i> - pus	≤0.5	1.0	Minor
<i>Pseudomonas</i> - pus	≥16.0	1.0	Very major error
<i>Pseudomonas</i> - sputum	≤0.5	1.0	Minor
<i>Acinetobacter</i> - urine	≤0.5	1.0	Minor

- a. Minor discrepancy is when there are differences in MIC values obtained by both the methods but no change in category of interpretation.
- b. Major discrepancy is when difference in MIC values cause difference in category of interpretation.

**DISCUSSION**

1. Carbapenem resistant Enterobacteriaceae are a majority of the isolates. *Klebsiella* (28.88%) causing UTI is the predominant isolate-sample wise followed by *E. coli* (18.88%) and *Pseudomonas* (13.33%) in urine. Study carried out by Marya et al. [8] showed similar findings of *Klebsiella* being the predominant isolate.
2. Urine forms the bulk of samples with carbapenem resistant gram negative isolates (67.77%). Study by Marya et al. [8] showed similar findings of UTI contributing to carbapenem resistant isolates.

3. In case of *Klebsiella pneumoniae* out of 40 isolates in our study, only 1 isolate had a discrepancy in MIC values and the MIC given by Vitek was ≥16 mcg/ml. We infer that in case of *Klebsiella pneumoniae*, reconfirmation by BMD needs to be done only in case of MIC ≥16 mcg/ml. More number of isolates will have to be studied to corroborate the above inference.
4. In case of *Enterobacter aerogenes*, only 3 isolates were studied and had no discrepancy. But the low number of isolates does not allow any conclusion to be made.
5. In case of *E. coli*, out of 20 isolates, 3 had discrepancy in the values of MIC, which was minor error as it did not change the category of interpretation. So reporting by Vitek 2 compact for them can be taken into consideration.

6. In case of *Acinetobacter*, 7 isolates were studied and had no discrepancies. Yen et al. [9] showed similar findings.
7. But because the outcome of colistin use is dependent on the exact value of colistin MIC, this testing will have to be continued.
8. Our study is limited by the fact that we do not have a single case of colistin resistance by BMD. We did not find any such study.

## CONCLUSION

1. The broth micro dilution (BMD) technique is reliable and is easy to use method for determining the MIC of Colistin. The results correlated with Vitek 2 compact except for 2 isolates which showed very major errors which indicates that in case of resistance to Colistin by Vitek, broth dilution method must be used for correlation and to recheck the result.
2. Also in case of Vitek 2 Compact showing susceptibility to Colistin, we can safely report those isolates without doing micro broth dilution as we did not encounter any isolates which gave susceptible on Vitek and resistant on micro broth dilution method.

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