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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. The Broth Microdilution (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 *Paenibacillus 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-β-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 Microdilution (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 bioMe´rieux and Liofilchem) exhibit [6]. Colistin has been traditionally reported by all automated systems like VITEK, Phoenix since many years. CLSI guidelines 2018 issued a correction as follows- the only approved MIC method for testing is broth microdilution 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.

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Study

We have undertaken this study to compare colistin susceptibility using BMD and Vitek to evaluate the discrepancies and further course of action.

Colistin susceptibility is done in our lab using MICROLATEST® marketed by Transasia in India. It is a broth microdilution 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* and *Acinetobacter* spp. are as follows as per CLSI 2018. Resistant: >=4 mcg/ml; Susceptible: <=2 mcg/ml

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

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* and *Acinetobacter* spp.

Recommendations for MIC determination of colistin (polymyxin E)

As recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group Published on www.eucast.org 22 March 2016 [4].

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 microdilution 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 methanesulfonate 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* [7].

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

2. Urine forms the bulk of samples with carbapenem resistant gram negative isolates (67.77%) (Table 2).

Table 1: The carbapenem resistant isolate distribution was as follows.

Isolate	N0. of isolates	% of isolates
Enterobacteriaceae:	64	71.11
Klebsiella pneumoniae	40	44.44
E. coli	21	23.33
Enterobacter aerogenes	3	3.33
Non-fermenters:	26	28.89
Pseudomonas aeruginosa	19	21.11
Acinetobacter baumannii	7	7.77
Total	90	100

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

Sample type	No. of isolates	% of isolates
Urine	61	67.77
Pus	10	11.11
Sputum	9	10
E.T. secretions	6	6.66
Blood	3	3.33
Bile	1	1.11
Total	90	100

3. *Klebsiella* causing UTI is the predominant isolate-sample wise followed by *E. coli* and *Pseudomonas* in urine (Table 3).

4. 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 (Tables 4 and 5).

Details of the discrepancy (Table 6):

- Minor discrepancy is when there are differences in MIC values obtained by both the methods but no change in category of interpretation.
- 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 Zilberberg 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 Zilberberg 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.

Table 3: The Organism distribution sample wise is as follows.

Sample type	*E. coli*	*Klebsiella pneumoniae*	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*
Urine	17	26	2	12	2
Pus	2	2	1	3	2
Sputum	1	7	0	3	0
ET secretions	0	3	0	1	2
Blood	0	2	0	0	1
Bile	1	0	0	0	0
Total	21	40	3	19	7

Table 4: MIC distribution in gram negatives by BMD is as follows.

Org	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
E. coli	21	21	20	-	-	-	1				
Klebsiella pneumoniae	40	39	40	-	-	-	-	-	-	1	-
Enterobacter aerogenes	3	3	3	-	-	-	-	-	-	-	-

Table 5: MIC distribution in gram negatives by BMD is as follows.

Organism	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
Pseudomonas aeruginosa	19	18	19	-	-	-	-	-	-	1	-
Acinetobacter baumannii	7	7	7	-	-	-	-	-	-	-	-

Table 6: Details of the discrepancy.

Isolate	Mic by vitek	Mic by bmd	Type of error
Klebsiella- Urine	>=16.0	0.25	Very major error
E. coli- Sputum	<=0.5	2	Minor
E. coli- Pus	<=0.5	1	Minor
E. coli- urine	<=0.5	1	Minor
Pseudomonas –et secretions	<=0.5	1	Minor
Pseudomonas- Urine	<=0.5	2	Minor
Pseudomonas- Urine	<=0.5	1	Minor
Pseudomonas- Urine	<=0.5	1	Minor
Pseudomonas- pus	<=0.5	1	Minor
Pseudomonas- pus	>=16.0	1	Very major error
Pseudomonas –sputum	<=0.5	1	Minor
Acinetobacter -urine	<=0.5	1	Minor

Minor discrepancy is when there are differences in MIC values

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. Tan 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 Microdilution (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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