Galore International Journal of Health Sciences and Research
Vol.4; Issue: 4; Oct.-Dec. 2019
Website: www.gijhsr.com
Original Research Article *P-ISSN: 2456-9321*

Colistin Susceptibility for Carbapenem Resistant Gram Negative Bacilli; Comparative Study of E-test and Vitek 2 Compact™ with Broth Microdilution

Ayushi Sharma[1], Mohit Agrawal[2]

[1]Postgraduate student, [2]Associate Professor,
Department of Microbiology, Mahatma Gandhi University of Medical Sciences and Technology, Jaipur

Corresponding Author: Mohit Agrawal

ABSTRACT

Purpose: Colistin remains part of the last line drugs used to treat multi-drug resistant Gram negative bacilli, like carbapenem resistant GNB. At present CLSI-EUCAST joint recommendations are for using Broth microdilution (BMD) as the reference method for testing colistin susceptibility. This study was undertaken to assess and compare commercially available susceptibility testing methods for MIC determination of colistin against the reference BMD.

Method: 510 CRGNB were included under the course of 6 months, from January 2019 to June 2019. Carbapenem resistance was detected by Vitek 2 Compact™ and the isolates were further processed for colistin susceptibility testing by E-test, Vitek 2 Compact™ (Biomerieux, France) and BMD.

Result: 32 (6.27%) isolates were found colistin resistant by the reference method BMD. *Klebsiella pneumoniae* (43.75%) was the predominantly isolated organism, followed by *Pseudomonas aeruginosa* (31.25%), *Enterobacter cloacae complex* (12.5%), *Acinetobacter spp.*(6%), *E.coli* (3%) and *Enterobacter aerogenes* (3%). Rates of essential agreement (EA) for E-test and Vitek 2 Compact™ were 66% and 85% respectively. Categorical agreement (CA) was found to be 73% or E-test and 96% for Vitek 2 Compact™. There was no very major error (VME) for Vitek 2 Compact™. E-test presented with 33% VME. While there was no major error (ME) for E-test, 4% of ME were noted for Vitek 2 Compact™.

Conclusion: The findings of this study showed substantial discordance between colistin susceptibility testing methods, with the overall reliability E-test being poor for MIC determination and reliability of Vitek 2 Compact™ was considered moderate when BMD was taken as the reference method.

Keywords: Colistin, Carbapenem, Gram negative bacilli, Broth microdilution, E-test

INTRODUCTION

The continuing emergence of multi-drug resistant organisms (MDROs) presents with an escalating burden on health care systems globally, contributing to increased morbidity and mortality. This rapid increase in resistance pattern coupled with the shortage of new antimicrobial agents has rekindled interest in the usage of older drugs like polymyxins for treating multi-drug resistant infections. Owing to nephrotoxicity and neurotoxicity, polymyxins initially fell out of favor among clinicians. [(1)] In recent years, with the surge in rates of carbapenem resistant infections, polymyxins are increasingly used in combination therapy for synergistic antimicrobial activity. [(2, 3)]

Polymyxins are polycationic peptides encompassing five chemically different compounds (Polymyxins A-E). Polymyxin B was first isolated in Japan, in 1949, derived from *Bacillus polymyxa*. Polymyxin E (Colistin) was obtained from *Bacillus polymyxa subspecies colistinus*. [(4)] Polymyxins B and E have been used in clinical practice since 1959, while polymyxin A, C and D are not used because of toxicity. Members of this class of antibiotics primarily act on the cell wall of

Gram negative bacilli, causing rapid changes in permeability of the cytoplasmic membrane, ultimately leading up to cell death.

Emergence of multi-drug resistance among clinically important Gram-negative bacteria has expedited the revival of colistin into clinical use. Conceivably, resistance to colistin has also emerged in Gram-negative pathogens such as *Acinetobacter spp., Pseudomonas aeruginosa, Escherichia coli and Klebsiella spp.* [(5)] mediated by chromosomal mutations as well as by genes such as mcr-1 and mcr-2 present on mobile elements. [(6,7)] This underscores the urgent need for standardized in vitro susceptibility testing both, for patient care and for epidemiological surveillance purposes. However, the testing has been a challenging task due to the inherent properties of colistin like its cationic nature, an affinity for plastic as well as a poor diffusibility in agar.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) have established colistin MIC breakpoints for *Acinetobacter spp.* And *Pseudomonas spp,* ≥2 µg/ml being resistant. EUCAST has also established breakpoints for Enterobacteriaceae with resistance observed at ≥2 µg/ml, while CLSI has to set those.

In 2016, the joint CLSI - EUCAST polymyxin breakpoint working group concurred that the ISO -20776 standard broth microdilution method (BMD) (Same methodology outlined in the CLSI M07 document 9) should be used for colistin MIC determination, and should be performed with colistin sulfate salt in plain polystyrene trays sans additives like polysorbate -80 (P80). The disk and gradient diffusion methods are currently not recommended.

The use of BMD in routine diagnostic laboratories is not feasible owing to large sample load. Hence a reliable and reproducible method for susceptibility testing is required. We compared Etest and Vitek 2 Compact™ against the reference broth microdilution method to provide with an alternate testing method.

MATERIAL AND METHOD

The test group in this study was carbapenem resistant Gram negative bacilli isolated from various clinical samples. Samples included in this study were obtained from different wards and outdoor patient department of a tertiary care hospital in Jaipur between the duration of January 2019 to June 2019. Clinical samples including blood, CSF, urine, respiratory secretions, swab from non-healing ulcers, pus/ wound swabs and any other sample from sterile body fluids were the specimen for our study. Susceptibility testing for carbapenems was done on Vitek 2 Compact™.

Carbapenem resistant Gram negative bacilli isolated were then processed further for colistin susceptibility.

As per CLSI recommendations, BMD was performed with cation-adjusted Mueller-Hinton broth (CA-MHB), a range of 2-fold dilutions of colistin (ranging from 0.125 to 8 µg/ml), and a final bacterial inoculum of 5 $x10^5$ CFU/ml in each well. MIC was defined as the lowest concentration of colistin at which no visible growth was obtained using CLSI recommended incubation conditions. [(8)]

According to the manufacturer's recommendations (HiMedia), E-test was performed by applying a bacterial inoculum of approximately 10^8 CFU/ml (turbidimetry of a 0.5 McFarland standard) suspended in 0.85% NaCl onto the entire surface of an MH agar plate using a sterile cotton swab. E-test strips containing a colistin concentration gradient (ranging from 0.016 to 256 µg/ml) were placed on the inoculated agar surface, and the MIC was determined after incubation for 16 to 24 h at 35±2°C. MIC is defined by the intersection of the lower part of the ellipse-shaped growth inhibition area with the test strip.

Vitek 2 Compact™ system (bioMerieux, France) uses plastic reagent cards that contain microliter quantities of

antibiotics and test media in wells. It tests colistin concentrations ranging from 0.5 to 16 µg/ml and observes turbidimetry to determine bacterial growth through a period of 4 to 10 h.

Data analysis:

The MIC results obtained by the methods in this study were analyzed by comparing them against the MIC obtained by reference method BMD as per CLSI guidelines. [(8)] Essential agreement (EA) was calculated as the percentage of isolates having MIC values within ±1 twofold dilution of the reference method. Categorical agreement (CA) was calculated as the percentage of isolates with results in the same category as the reference method. Very Major Error (VME), an isolate resistant by the reference method, but susceptible by the test method, represented false susceptibility. Major error (ME), an isolate susceptible by the reference method, but resistant by the test method, represented false resistance. Minor error (MiE), defined as the isolate being resistant or susceptible by the reference method, but intermediate by the test method, was precluded as there is no intermediate category for colistin. Reliability of individual tests falling in the outlined breakpoints was determined according to the following criteria:

It was considered high if both EA and CA were >90%, moderate if either EA or CA is >90%, low if both EA and CA were <90% and there were acceptable errors (<2% VME, <5% ME), and poor if the errors were unacceptable, irrespective of EA and CA.

RESULT

Table 1. Location wise distribution of Carbapenem resistant Gram negative bacilli

IPD	OPD	Total
469 (91.96%)	41 (8.04%)	510 (100%)

Results of individual AST methods for colistin:

With Broth microdilution method, 32 isolates from 510 CRGNB yielded an MIC of ≥2 µg/ ml, that is resistant. All isolates tested by E-test yielded MICs ≤2 µg/ ml, that is sensitive. With Vitek 2 Compact, 36 isolates yielded an MIC of ≥2 µg/ ml, that is resistant.

Table 2. Specimen wise distribution of Carbapenem resistant Gram negative bacilli isolates.

Carbapenem resistant Gram negative bacilli Specimen	Count
ET sec.	144 (28.23%)
Urine	141 (27.64%)
Pus swab	53 (10.39%)
Swab	40 (7.84%)
Sputum	30 (5.88%)
Blood	30 (5.88%)
Pus	24 (4.70%)
BAL	10 (1.96%)
CSF	8 (1.56%)
Drain	7 (1.37%)
Ascitic fluid	5 (0.98%)
Tissue	5 (0.98%)
Fluid	4 (0.78%)
Pleural fluid	3 (0.58%)
Endometrial fluid	3 (0.58%)
Central line tip	2 (0.39%)
Bile	1 (0.19%)
Total	510 (100%)

Table 3. Species wise distribution of Carbapenem resistant Gram negative bacilli

Carbapenem resistant Gram negative bacilli	Count
Acinetobacter baumanii complex	162 (31.76%)
E. coli	161 (31.56%)
Klebsiella pneumoniae	113 (22.15%)
Pseudomonas aeruginosa	54 (10.58%)
Enterobacter cloacae complex	17 (3.33%)
Enterobacter aerogenes	3 (0.58%)
Total	510 (100%)

Table 4. Clinical speciality wise distribution of patients presenting with Carbapenem resistant Gram negative bacilli infections

Clinical speciality (CRGNB)	No. of isolates (%)
Critical ICU	78 (15.29%)
Med. ICU	59 (11.56%)
Tracheostomy ward (ICU)	42 (8.3%)
HDU	30 (5.88%)
MMW	27 (5.29%)

Table 5. Location wise distribution of colistin resistant organisms

IPD	OPD	Total
28 (88%)	4 (12%)	32 (100%)

Table 6. Distribution of colistin resistant organism in different specimens

Colistin resistant organisms in different specimen	Count
Urine	11 (34.37%)
ET secretions	6 (18.75%)
Pus swab	4 (12.5%)
Sputum	3 (9.3%)
Swab	2 (6.25%)
Pleural fluid	2 (6.25%)
BAL	1 (3.12%)
Blood	1 (3.12%)
Endometrial fluid	1 (3.12%)
Pus	1 (3.12%)
Total	32 (100%)

Table 7. Species wise distribution of colistin resistant organisms

Colistin resistant organisms	Count
K.pneum.pneumoniae	14 (43.75%)
Ps.aeruginosa	10 (31.25%)
Ent.cloacae complex	4 (12.5%)
Acinetobacter spp	2 (6.25%)
Esch.coli	1 (3.12%)
Ent.aerogenes	1 (3.12%)
Total	32 (100%)

Table 8. : Results of individual AST methods for colistin

Antimicrobial susceptibility testing method for colistin	Count
Broth microdilution	32
E- test	0
Vitek 2 Compact	36

Comparison of different MIC testing methods for colistin susceptibility:

Taking BMD as the reference method, EA for E-test was 66%. For Vitek 2 Compact, EA was 85%.

There was no VME noted for Vitek 2 Compact, while it was 33% for E-test.

Vitek 2 compact yielded ME of 4% while there were no ME for E-test.

Table 9: Comparative study of E-test and Vitek-2 with Broth microdilution

AST Method	Essential agreement (EA)	Categorical agreement (CA)	Very major errors (VME)	Major errors (ME)
E-test	66%	73%	33%	0
Vitek 2 Compact	85%	96%	0	4%

The overall reliability of E-test method were poor for testing colistin susceptibility. Reliability of Vitek 2 Compact was considered moderate when BMD was taken as the reference method.

DISCUSSION

The emerging resistance in Gram negative bacilli poses necessity to find a reliable susceptibility testing method for last line drugs like colistin. Various studies have compared different susceptibility testing method against the reference broth microdilution method. Currently CLSI-EUCAST joint group recommends broth microdilution method in plain polystyrene microtitre plates, Cation adjusted Mueller Hinton broth without any Polysorbate 80 (P80) supplementation.

The E-test is a simple and accurate alternative method for the susceptibility testing of colistin but owing to poor diffusion of colistin in the agar medium, one to four percentage of false susceptible result but no false-resistant results were obtained in various studies conducted by Loe-Ten-Foe JR and Behera et al. [9, 10] The highest rate of very major errors (VMEs) reported for colistin after an E-test is 41.5% [11] The present study echoed the same concern of false susceptibility, where no false resistance was observed.

Vitek 2 Compact showed moderate agreement with BMD in the present study, with CA of 96%. This is in agreement with two prior studies conducted byPiewngam, P., and P. Kiratisin. (2014) [12] and Dafopoulou, K. et al (2015) [13] that observed moderate agreement of Vitek with BMD despite CA of 94% and 90%, respectively. Although authors of the latter study [13] have recommended the use of Vitek for AST determination of polymyxins for CRAB, concerns regarding the unreliability of Vitek in view of heteroresistance in *A. baumannii* have been raised [9] and hence caution is advised while interpreting the results.

Tan et al (2007) [14] reported results for colistin susceptibility testing for *Acinetobacter* spp. isolates, showing categorical agreement between Vitek 2 and agar dilution, with no false-resistance reported by Vitek 2. This contradicts the present study where 4% of false resistance was observed by Vitek 2 Compact

As opposed to the present study, where there was discordance between E-test and BMD, Tan et al (2007) [15] when testing mixed Gram negative bacilli reported categorical agreement between agar dilution and E-test as 87%, and between agar dilution and Vitek 2 as 82%. Based on the data obtained, Tan et al reported that the Vitek 2 system was unreliable for detecting colistin resistance, and results obtained by E-test may require confirmation by a standard MIC susceptibility testing method.

Arroyo et al (2005) [16] compared the E-test to the broth microdilution method for testing the susceptibility of 115 clinical isolates of *Acinetobacter baumannii* to colistin. Twenty-two (19.1%) strains were resistant to colistin and 93 (80.8%) strains were susceptible according to the reference broth microdilution method. A categorical agreement of 98.2% was found, with only two (1.7%) very major errors. Agreement within 1 twofold dilution between the E-test and the broth microdilution was 16.5%. Complete agreement was found for the strains for which MICs fell within the range of 0.25 to 1 µg of colistin/ml. However, there was poor concordance, particularly in extreme dilutions with higher MICs by the E-test method. This was in conjuncture to the present study.

Goldstein et al (2007) [17] evaluated 170 clinical isolates of Gram-negative including a total of 64 P. aeruginosa (12 colistin-resistant strains) and compared E-test with agar dilution (reference method) for testing susceptibility to colistin. MICs of < 4 mg/l were considered to indicate susceptibility to colistin. E-test showed 91% of agreement (± 2-fold dilution) in comparison with the reference method, as opposed to our study where we observed 66% EA for E-test. Piewngam and Kiratisin (2014) [12] have reported moderate agreement between BMD and colistin E-test with EA of 95% and no errors. Hindler and Humphries (2013) [18] have reported poor agreement between BMD-polysorbate 80 and three types of colistin E-strips (from different manufacturers) with EA of 61% and 14% VME. Dafopoulou et al. (2015) [11] have reported VMEs to the tune of 35% when colistin E-test was compared with BMD. In the present study, EA of 66% and CA of 73% for E-test was noted.

CONCLUSION

Out of the 510 carbapenem Gram negative bacilli isolates, 32 (6.27%) were found colistin resistant when broth microdilution was taken as the reference standard.

The findings of the present study showed substantial discordance between testing methods, with the overall reliability of E-test being poor for testing colistin susceptibility and reliability of Vitek 2 Compact was considered moderate when BMD was taken as the reference method.

REFERENCES

1. Landman D, Georgescu C, Martin DA, Quale J. 2008. Polymyxins revisited.Clin Microbiol Rev 21:449–465.
2. Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS. 2014. Antibiotic treatment of infections due to carbapenem-resistant enterobacteriaceae: systematic evaluation of the available evidence. Antimicrob Agents Chemother 58:654 – 663.
3. Nabarro LEB, Veeraraghavan B. 2015. Combination therapy forcarbapenem-resistant Enterobacteriaceae: increasing evidence, unanswered questions, potential solutions. Eur J Clin Microbiol Infect Dis34:2307–2311.
4. Komura S, Kurahashi K. Partial purification and properties of L-2,4-diaminobutyric acid activating enzyme from a polymyxin E producing organism, *J Biochem (Tokyo)*, 1979, vol. 86 (pg. 1013 21)
5. Gales AC, Jones RN, Sader HS2011. Conte mporary activity of colistin and polymyxin B against a worldwide collection ofGram-negative pathogens: results from the SENTRY Antimicrobial Surveillance Program (2006–09). J Antimicrob Chemother 66:2070–2074. doi:10.1093/jac/dkr239.
6. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, DongB, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, LiuJH, Shen J (2015) Emergence of plasmid mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16(2):161–168
7. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H,Malhotra-Kumar S (2016) Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli*, Belgium, June 2016. EuroSurveill 21(27).

8. CLSI M23-A2 202: Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline-Second Edition
9. Lo-Ten-Foe JR, de Smet AM, Diederen BM, Kluytmans JA, van Keulen PH(2007) Comparative evaluation of the VITEK 2, disc diffusion, etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter Cloacae* and *Acinetobacter baumannii* strains. Antimicrob Agents Chemother 51(10): 3726–3730.
10. Behera B, Mathur P, Das A, Kapil A, Gupta B, Bhoi S, Farooque K, Sharma V,Misra MC. 2010. Evaluation of susceptibility testing methods for polymyxin. IntJ Infect Dis 14:e596–e601.
11. Dafopoulou K, Zarkotou O, Dimitroulia E, Hadjichristodoulou C, GennimataV, Pournaras S, Tsakris A. 2015. Comparative evaluation of colistin susceptibility testing methods among carbapenem-nonsusceptible Klebsiella pneumoniae and Acinetobacter baumannii clinical isolates. Antimicrob Agents Chemother 59:4625– 4630.
12. Piewngam, P., and P. Kiratisin. 2014. Comparative assessment of antimicrobial susceptibility testing for tigecycline and colistin against *Acinetobacter baumannii* clinical isolates, including multidrug-resistant isolates. Int. J.Antimicrob. Agents 44:396–401.
13. Dafopoulou, K., O. Zarkotou, E. Dimitroulia, C. Hadjichristodoulou, and V.Gennimata. 2015. Comparative evaluation of colistin susceptibility testing methods among carbapenem nonsusceptible *Klebsiella pneumoniae* and *Acinetobacter baumannii* clinical isolates. Antimicrob. Agents Chemother.59:4625–4630.
14. Tan TY, Ng SY. 2007. Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin. Clin Microbiol Infect 13:541–544.
15. Tan TY, Ng LS, Poh K. Susceptibility testing of unconventional antibiotics against multiresistant *Acinetobacter* spp. by agar dilution and Vitek 2. DiagnMicrobiol Infect Dis 2007;58:357-61.21
16. Arroyo LA, Garcia-Curiel A, Pachon-Ibanez ME, Llanos AC, Ruiz M, Pachon J,Aznar J (2005) Reliability of the E-test method for detection of colistin resistance in clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol. 43(2):903–905.
17. Goldstein FW, Ly A, Kitzis MD: Comparison of Etest with agar dilution for testing the susceptibility of *Pseudomonas aeruginosa* and other multidrug-resistant bacteria to colistin. J Antimicrob Chemother 2007,59:1039-40.
18. Hindler JA, Humphries RM (2013) Colistin MIC variability by method for contemporary clinical isolates of multidrug-resistant gram-negative bacilli. J Clin Microbiol 51(6):1678–1684.

How to cite this article: Sharma A, Agrawal M. Colistin susceptibility for carbapenem resistant gram negative bacilli; Comparative study of E-test and Vitek 2 Compact™ with Broth microdilution. Galore International Journal of Health Sciences & Research. 2019; 4(4): 110-115.

******