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Evaluation of Vitek2 and Colistin broth disk elution test in comparison with Micro broth dilution for Susceptibility testing of Colistin among Carbapenem Resistant Enterobacterales.

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Abstract

Colistin is the last expedient for treating the infections caused by Carbapenem resistant Enterobacter ales (CRE). Expanding usage of colistin as led to development of Colistin resistance. This urges for a need of reliable and accurate testing method that can be adopted as routine for susceptibility testing of Colistin. The innate cationic property of colistin molecules is associated with complexities. Hence evaluation of testing method is needed for detection of colistin resistance. Present study is done to evaluate the results yielding from Vitek2 and CBDE in comparison with rBMD. About 200 CRE isolated from clinical specimens were tested, results of Vitek2, CBDE was compared with rBMD. In comparison with BMD Categorical agreement (CA) of Vitec 2 was 83% with major error of 17% .CA of CBDE was 99% with 1% of very major error. Since the resistance to colistin is increasing accurate reporting of Colistin MIC by a validated method becomes important. So, we would recommend CBDE for routine testing of Colistin susceptibility.

Keywords: Carbapenem resistant Enterobacterales, Colistin broth disk elution, rBMD- reference broth microdilution, Categorical Agreement.

Introduction

Emergence of MDR (Multidrug resistant) microorganisms especially Carbapenem resistant Enterobacterales (CRE) are alarmingly being reported worldwide. Because of the restricted treatment choice, it's been challenging to treat the infections caused by these CRE organisms. Polymyxins (colistin or Polymyxin B and Polymyxin E) antimicrobials remain the last resource for treating the infections caused by these Carbapenem resistant gram-negative bacteria [(1)]. Acquired resistance to these polymyxins was reported less in the past but in the present situation because of its expanding usage both clinically and non -clinically resistance to colistin is more frequently being reported [2]. Hence it becomes crucial that clinical microbiology laboratories should be able to appropriately identify organisms which are resistant to colistin, so that such kind of drugs are cautiously used for treatment [3,4]. This further signifies the need of requirement of a standardised antimicrobial susceptibility test (AST) for colistin, that helps for both patient-care and for surveillance purposes. Presently Broth Micro dilution (BMD) is the only standard reference method for colistin AST according to CLSI [5] CLSI is also evaluated other alternative MIC based methods such as colistin broth disk elution test (CBDE) & colistin agar test (CAT) as accurate as reference BMD [4]. Although BMD is a reference method it is difficult to be done on routine basis because of its laborious nature. In

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most of the laboratories, MIC of colistin is determined and reported by Vitek-2 automated antibiotic susceptibility test (AST) system, even though it is not an approved method by CLSI [5]. In view of this present study was taken up to evaluate the results of Vitek-2, CBDE in comparison with rBMD so that we can analyse if we can continue reporting Colistin MIC from Vitek-2 AST or adapt new test like CDBE.

Materials and Methods

A cross sectional analytical study was conducted in department of Microbiology, JSS medical college and hospital, about 200 clinical isolates yielded from routine clinical specimens , such as blood, endotracheal aspirate, sputum, sterile fluids (bile, ascitic fluid, CSF) and exudate specimens which were Enterobacterales & Carbapenem resistant were included in the study . Other organisms from the Enterobacterales which are intrinsically resistant to colistin such as *Proteus, Serratia, Morganella* and *Providencia* were excluded, also clinical isolates from stool samples & clinical isolates isolated from same patient were also excluded from the study. The study involved the specimens that were collected from the samples that were routinely received in Microbiology laboratory for evaluation & since no intervention was needed informed consent was not taken , by application of statistical tool 200 clinical isolates which were resistant to Carbapenem were subjected to broth micro dilution (BMD), colistin broth disk elution and vitek-2 AST system . Results were noted and analyzed. Reference in-house BMD was performed according to standard operating protocol issued by National Programme on Antimicrobial Resistance Containment National Centre for Disease Control, India, August 2020 , Cation adjusted Mueller-Hinton broth (90922) was used for performing CBDE , discs were procured from Oxoid™ & test was performed according to CLSI 2020, M100 document using appropriate control strains. Thermo Scientific company and colistin sulphate salt (C4461) was procured from Sigma Aldrich company. If susceptibility result of the isolate done by the test method (CBDE and vitek-2) is same as the reference standard method (BMD), the test method is said to be categorically agreed with that of the reference method, if not it is categorically disagreed. Again Categorical disagreement is classified into Very Major, Major and Minor errors. If the test method shows sensitive category and the reference method is resistant, it is very major error. If the test method shows resistant category and the reference method is sensitive, it is major error. If the test method is intermediate category and the reference method is either sensitive or resistant category, it is said to be Minor error. As EUCAST does not give any intermediate breakpoint for colistin, Minor errors is not applicable for colistin .

Results

Among 200 clinical isolates the most common organism isolated was *Klebsiella pneumoniae* (112), followed by Escherichia coli 71 and Enterobacter cloacae 13 (**Table 2)**. Colistin MIC distribution of 200 clinical isolates by reference BMD method. 23 isolates showed resistance and organism that exhibited highest resistance was by Klebsiella *pneumoniae* **(Table 30).** 92 isolates showed MIC value of ≤1 by both CBDE & MBD, 11 isolates showed MIC of 2 in both CBDE and reference BMD, 21 isolates showed MIC of ≥4 in both CBDE & reference BMD , 2 isolates showed MIC of 2 in CBDE & 4 MIC value in reference BMD which accounts for very major error . Categorical agreement of CBDE with respect to reference BMD was 99% **(Table 4)** 135 isolates yielded MIC of ≤1 in vitek-2 & reference BMD , 23 isolates showed MIC of ≥ 4 in both Vitek 2 and reference BMD , 13 isolates showed MIC of 4 in Vitek-2 and 1 in reference BMD, 12 isolates yielded MIC value of 8 in Vitek 2 and 2 in reference BMD, 9 isolates showed MIC of 16 in Vitek 2 and 2 in reference BMD, in total 34 isolates were resistant in test method that is Vitek -2 and sensitive in reference BMD which indicates major error. 83% of categorical agreement in comparison of test method that is Vitek 2 with reference method that is BMD.

Tables & Figures

Table 1: Colistin interpretative breakpoints according to the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines- 2021 was used for interpretation of the results.

Organism/groups	CLSI 2021			EUCAST 2021		
	S	I	R	S	R	ATU
Enterobacterales	-	≤2	≥4	≤2	>2	-
Pseudomonas aeruginosa	-	≤2	≥4	≤2	>2	4

Organism/groups	CLSI 2021			EUCAST 2021		
Pseudomonas spp	-	-	-	≤2	>2	4
Acinetobacter baumannii complex	-	≤2	≥4	≤2	>2	-
Acinetobacter spp	-	-	-	≤2	>2	-
Non-Enterobacterales*	-	-	-	-	-	-

**Other non-fermenting Gram-negative bacilli except Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia and Burkholderia cepacia*

ATU- area of technical uncertainty; S- Susceptible; I- intermediate; R-resistant

Table 2: Distribution of carbapenem resistant Enterobacterale isolates

Organism	Number of isolates tested
Klebsiella pneumoniae	112
Escherichia coli	71
Enterobacter cloacae	13
Citrobacter species	04

Table 3: Colistin MIC distribution for 200 isolates with reference BMD method is shown in [Table-3]

Organism	No. of isolates tested	MIC range (µg/mL)							
		0.125	0.25	0.5	1	2	4	8	16
Klebsiella pneumoniae	112	20	10	30	16	18	8	4	6
Escherichia coli	71	20	21	22	3	2	3	0	0
Enterobacter cloacae	13	2	3	4	1	1	2	0	0
Citrobacter species	04	1	1	2	0	0	0	0	0

Table 4: Colistin MIC distribution of CBDE with reference to MBD method

MIC of CBDE	MIC of MBD								
	MIC range (µg/mL)	0.125	0.25	0.5	1	2	4	8	16
	≤1	23	18	30	11	10	0	0	0
	2	20	17	28	9	11	2*	0	0
	≥4	0	0	0	0	0	11	4	6

**Indicated very major error*

Table 5: Colistin MIC distribution of Vitek-2 with reference to MBD method

MIC range (µg/mL)	0.125	0.25	0.5	1	2	4	8	16
≤0.5	20	16	30	3	0	0	0	0
1	18	19	25	4	0	0	0	0
2	5	0	3	0	0	0	0	0
4	0	0	0	13*	0	8	3	0
8	0	0	0	0	12*	5	1	4
16	0	0	0	0	9*	0	0	2

* Indicates major error

Discussion

There is re-emergence of usage of polymyxin drugs over the past as last agent for treatment of infections caused by multi drug resistant gram-negative rods. Although polymixins being considered as last option of treatment it should be used carefully since even in optimal doses they are highly nephrotoxic and neurotoxic , hence knowing the MIC of infecting organism is of clinical importance so that accurate report is communicated to the treating physician which ensures that such kind of drugs are discreetly used [6]. CLSI and EUCAST [7,8] in the year 2017 together recommended rBMD as the reference test for Colistin testing. But it has its own limitations since the procedure is tedious and also difficult to use as routine test for detection of colistin resistance in resource limited settings , so there is a need of an alternative method which is more amicable to be performed on routine basis . Many laboratories are reporting colistin MIC by using Vitek-2 system which is not an approved method by CLSI . Apart from rBMD CLSI is also recommended CBDE and CAT as alternative method for colistin MIC reporting , present study focuses on evaluation of results of colistin MIC with VITEK -2 system & CBDE in comparison with rBMD to evaluate which is the better method that can be adopted on routine basis for testing the colistin susceptibility. Reference micro broth dilution (MBD) was performed on 200 Carbapenem resistant Enterobacterales which were isolated from clinical specimens among which 11.5 % were resistant to colistin . Various other studies also showed similar kind of resistant pattern, Klebsiella pneumoniae showed the highest resistance to colistin among other Enterobacterales which was around 9% similarly In the year 2019 Walia k et al also reported around 8 % of colistin resistance in *K. pneumoniae* causing hospital-acquired infections 12.6% of klebsiella species isolated from urinary tract infection were colistin resistant in study conducted by Jain S. et al., (2018) [10]. 20.4 % of colistin resistance were reported in 2020 by Sarumathi D et al., [11]. High resistance to colistin was noted in many other studies conducted by authors like L. Bardet et al., in 2019 reported 63.4% of colistin resistance among gram negative rods [13]. Qadi M, et al., (2021) has reported 41% of Enterobacterales that were resistant to colistin [12]. Capone A, et al. reported around 36.1% of colistin resistance that were carbapenem resistant klebsiella pneumoniae in 2012 [14]. CBDE was performed the results were compared with reference BMD. Based on EUCAST 2021 guidelines Categorial agreement was analysed. Essential agreement was not analysed as CBDE was performed only in 3 dilutions whereas BMD was performed in 8 dilutions. As shown in **(Table 4).** 2 of the isolates showed MIC of 2 in CBDE & MIC value of 4 in reference BMD which is very major error, categorical agreement of CBDE with reference BMD was 99%. In a study conducted by Simner PJ, et al; which was a 2-site evaluation of CBDE method conducted in 2019, in this study in both the evaluation centre CBDE had 100% categorical agreement in comparison with reference BMD method (14). Similar findings were observed by Humphries RM et al, (2019) in which they reported 97.9% categorial agreement compared to the reference MIC and reported 9 VME (3.2%). Dalmolin TV et al., conducted a study in two different research centres in Brazil, in his study he reported 91.18% of CA and 4.95% of VME with CBDE compared to reference method (15).

As mentioned in (**Table 5)** 13 isolates with MIC value of 4 obtained by Vitek-2 showed MIC value of 1 in reference BMD, 12 isolates that yielded MIC of 8 in Vitek 2 showed MIC of 2 in reference BMD, 9 isolates with MIC of 16 resulted from Vitek 2 showed MIC value of 2 in reference BMD, 34 isolates totally were resistant in test method that is Vitek -2 and sensitive in reference BMD .Categorical agreement between Vitek-2 (Test method) in comparison with reference method(BMD) was 83% with 17% of major error and no very major error , many studies have been done for evaluation of Vitek-2 automated method for testing of colistin susceptibility. In a study conducted by Ka Lip Chew et al Similar categorical agreement was seen between Vitek -2 and BMD, which was < 90 % with very major error of 36% & no major error (17). In a study conducted by Jerome R. Lo-Ten-Foe et al vitek-2 had high level of categorical agreement in comparison with BMD, and he concluded that Vitek -2 is a reliable alternative tool for detection of colistin susceptibility and he specified that it's a good tool for detection of colistin susceptibility only in isolates that do not exhibit hetero resistance (18).

N. Pfennig Werth et al in his study Vitek 2 had 90.5% of categorical agreement with BMD and 31% of very major error with no detection of false resistance (19). Another study done by Salima Qamar et al the categorical agreement between Vitek 2 and BMD was 100% (20).

In our current study done for evaluation of alternative method for routine colistin testing Vitek 2 showed 83% of categorical agreement 17% of major error was detected in our study that is resistant in test method (Vitec 2) and sensitive in reference method (MBD) which indicates the detection of false resistance to colistin. And to be noted that there was no very major error detected by Vitec 2compared to BMD in our study. Whereas CBDE had very good categorical agreement of 99% with only 1 % of very major error and no major error compared to rBMD.

Conclusion

As per our study Vitec 2 detected more of false resistance to colistin but no false susceptibility was seen. Parallelly CBDE yielded good categorical agreement with only 1% of false susceptibility. Hence Vitek2 can be used routinely for testing of Colistin susceptibility but if colistin resistance is yielded by Vitek 2 then that isolate needs to be retested by one more method. CBDE can be used as alternative to vitek2 so that the isolate susceptibility will be double confirmed and false resistance will not be reported. This further avoids the unnecessary usage of other antibiotics like Fosfomycin,Tigecycline & other drugs that are used for treating colistin resistant infections.

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