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Evaluation of Colistin Broth Disk Elution and Colistin Agar Test: A study from Tertiary Care Hospital, South India

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Abstract

Enterobacterales particularly which are resistant to carbapenem group of antibiotics (CRE) are precariously being reported worldwide. Last option for treating the infections caused by CRE are polymyxin E (Colistin) and polymyxin B. Resistance to polymyxins is on higher side because of its increased usage both clinically and non-clinically. In vitro evaluation tests for susceptibility of colistin is associated with lot of complexities due to its innate cationic properties . Hence it is essential for all diagnostic laboratory to standardize colistin testing method, so the present study was undertaken to evaluate the results of colistin broth disk elution (CBDE) and colistin agar test (CAT) in comparison with the reference broth microdilution (rBMD). About 100 CRE clinical isolates were tested, results of CBDE & CAT was compared with rBMD. Categorical agreement (CA) of CBDE was 98% with 2% of very major error (VME), CA of CAT was 99% with 1% of VME in comparison with rBMD. Because of increasing colistin resistance it is crucial to report colistin MIC with a validated method, so we would like to recommend CAT test for routine MIC reporting of colistin since it is feasible test.

Keywords: Carbapenem-resistant Enterobacterales, colistin broth disk elution test, Colistin agar test, reference broth microdilution, Categorical agreement, Very major error, Major error

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INTRODUCTION

Emergence of multidrug resistant (MDR) microorganisms especially carbapenem resistant Enterobacterales (CRE) are alarmingly being reported worldwide. Old polycationic peptides like polymyxin E (Colistin) and polymyxin B have now regained its importance as last resort treatment for CRE.[1,2] Acquired resistance to polymyxin group of antibiotics was reported less frequently in the past, but now in present situation resistance to colistin is more frequently reported since there is increased usage of colistin both clinically and non -clinically. Hence, optimization of *in vitro* polymyxin susceptibility testing is now an essentiality for both patient treatment and for surveillance purposes.[2]

In vitro susceptibility testing of polymyxins, is challenging because of its intrinsic complex property such as the multicomponent composition, their poor capacity of diffusion into the agar, their property of binding to plates, polysorbate 80 (P-80) effectiveness, their capacity to develop heteroresistance.[4] Hence, disk diffusion, which is the most commonly performed AST in clinical diagnostic laboratories, could not be standardized for testing polymyxins.[1-3]

Till 2019, broth microdilution (BMD) was the only option available for AST of colistin, from Clinical and Laboratory Standards Institute (CLSI), but since 2020, CLSI has also approved colistin broth disk elution (CBDE) and colistin agar test (CAT) (5). BMD could not be implemented routinely, as it was labor-intensive procedure. Whereas, both CBDE and CAT can be implemented for testing colistin susceptibility for clinical diagnostics, as it is comparatively easy to perform.[1] Also to note, there is differences in recommendation between CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) regarding clinical breakpoints, which has been depicted in Table 1.[5,6]

It is essential for all clinical diagnostic laboratory to standardize colistin testing method considering the resources available and this in turn will aid clinicians to use the drug efficiently for treatment purposes. In view of this, the present study was undertaken to evaluate the results of CBDE, CAT in comparison with the reference BMD.

MATERIALS AND METHODS

A comparative analytical study was conducted in Microbiology laboratory, tertiary care hospital, Mysore, South India. Sample collection was done from January to April 2021. During this period, about 100 clinical isolates derived from routine clinical samples, such as blood, endo-tracheal aspirate, sputum, sterile body fluids (bile, ascitic fluid, CSF) and exudate specimens that were Enterobacterales which were carbapenem resistant were included in the study. Other organisms from the Enterobacterales family which are intrinsically resistant to colistin such as *Proteus* species, *Serratia* species, *Morganella* species and *Providencia* species were excluded, also clinical isolates from stool samples and isolates that were repeatedly isolated from the same patient were also excluded from the study.

The study was carried out on clinical isolates, derived from clinical samples which was sent for routine diagnostic evaluation to the hospital laboratory. As there was no intervention involved, informed consent was not taken from the patients. All 100 study clinical isolates were subjected to broth microdilution (BMD), colistin

Table 1. Colistin interpretative breakpoints according to the CLSI 2021 and EUCAST- 2021

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

broth disk elution (CBDE) and colistin agar test (CAT). Cation adjusted Mueller-Hinton broth was used while performing both CAT and CBDE.BMD was considered as gold standard and results of CBDE and CAT were compared with BMD, by applying required statistical tools.

Reference in-house BMD was performed on a polystyrene microtitre plate according to standard operating protocol issued by National Programme on Antimicrobial Resistance Containment National Centre for Disease Control, India, August 2020.[7] Cation adjusted Mueller-Hinton broth (90922) and colistin sulfate salt (C4461) was procured from Sigma Aldrich company. Test was performed using appropriate control strains. For quality control, ATCC 25922 *Escherichia coli* and NCTC *Escherichia coli* 13846 (MCR-1 positive) was used as recommended by EUCAST.CBDE and CAT was performed according to CLSI 2020, M100 document and colistin disk were procured from Oxoid™, Thermo Scientific company and colistin sulfate salt (C4461) was procured from Sigma Aldrich company.

Interpretation

If AST result of the isolate done by CBDE and CAT is similar to the reference BMD, then test method is considered to be categorically in agreement with the standard reference method, if not then it is considered as categorically disagreed. Categorical disagreement will be categorized further into very major error, major error, and minor error. In case the test is in sensitive category and the reference method is resistant, it is reported as very major error. If the test method is in resistant category and the standard reference method is in sensitive category, it is told as major error. If the test method is in intermediate category and the reference method is either insensitive or resistant category, it is termed as minor error. As EUCAST does not give any intermediate breakpoint for colistin, Minor errors is not applicable for colistin.[8]

Table 2. Distribution of carbapenem resistant Enterobacterales isolates

Organism	Number of isolates tested (%)
Klebsiella pneumoniae	49
Escherichia coli	41
Enterobacter cloacae	06
Citrobacter species	04

RESULTS

Among the 100 consecutively collected Enterobacterales (CRE) isolates, blood culture isolates accounts for 15%, endotracheal aspirate 35%; exudate samples 38% and sterile body fluid isolates is 12%. Among 100 clinical isolates, *Klebsiella pneumoniae* (49) was the most commonly isolated organism [Table 2]. Colistin MIC distribution for 100 isolates with rBMD method is shown in [Table 3].

As shown in Table 4, 69 isolates showed MIC ≤1 and 9 isolates showed MIC ≥4 in both test and reference method. 20 isolates showed MIC of 2 in both reference and test method, whereas 2 isolates showed MIC of 2 in test method and MIC of 4 in reference method, which accounts for very major error in CBDE with respect to reference method. So categorical agreement (CA) of CBDE was 98% with reference BMD.

As shown in Table 5, 69 isolates showed MIC ≤1 and 10 isolates showed MIC ≥4 in both test and reference method. 20 isolates showed MIC of 2 in both reference and test method, whereas 1 isolate showed MIC of 2 in test method and MIC of 8 in reference method, which accounts for very major error in CAT with respect to reference

Table 3. Colistin MIC distribution of reference MBD method

Organism	No. of isolates tested	MIC range (µg/mL)							
		0.125	0.25	0.5	1	2	4	8	16
Klebsiella pneumoniae	49	13	5	17	2	3	6	2	1
Escherichia coli	41	20	6	9	2	3	1	0	0
Enterobacter cloacae	06	1	1	2	0	1	1	0	0
Citrobacter species	04	1	1	2	0	0	0	0	0

Table 4. Colistin MIC distribution of CBDE with reference MBD method

	MIC range (µg/mL)	MIC of MBD							
		0.125	0.25	0.5	1	2	4	8	16
MIC of CBDE	≤1	32	12	21	4	0	0	0	0
	2	3	1	9	0	7	2*	0	0
	≥4	0	0	0	0	0	6	2	1

*Indicated very major error.

Table 5. Colistin MIC distribution of CBDE with reference MBD method

	MIC range (µg/mL)	MIC of MBD							
		0.125	0.25	0.5	1	2	4	8	16
MIC of CAT	≤1	32	12	21	4	0	0	0	0
	2	3	1	9	0	7	0	1*	0
	≥4	0	0	0	0	0	8	1	1

*Indicated very major error.

method. So categorical agreement (CA) of CAT was 99% with reference BMD.

In our organism wise analysis of very major error in CBDE and CAT in comparison to reference method (Table 4 and 5), we found that both errors were seen in *Klebsiella pneumoniae.*

DISCUSSION

Colistin is the last resource available for treating severe infections caused by MDR organisms. Over the last few years, the usage of colistin is increased by about 10-fold and hence rise in resistance is alarmingly noted. It is vital for Microbiology laboratory to have a feasible method for routine testing of colistin among the clinical isolates, so that they can guide clinicians in choosing colistin when indicated.[2,7-9]

Till 2019, only BMD was the approved method for testing by standard international and national guidelines. As there are lot of challenges in performing BMD routinely, many laboratories just relied on Vitek-2 system AST results for reporting it to clinicians, even though automated BMD by VITEK-2 is not approved by any international or national institutes. Hence it becomes vital to explore a feasible test which can implemented on a daily routine basis, so that appropriate AST of colistin reaches the clinicians. CBDE and CAT has been studied extensively at international platform, after CLSI has approved it for testing colistin, but each of these methods has its own merits and demerits which needs to be studied in Indian setting tertiary care hospital so that one these tests can be used routinely.

Reference BMD was performed for 100 study isolates of CRE, and it was noted that 11% isolates were colistin resistant. Studies done by various other authors also shows similar resistance pattern, Walia, K et al.[2] reported the prevalence of colistin resistance in *K. pneumoniae* causing hospital-acquired infections to be 8%[2]; Jain S. et al.[10] reported colistin resistance of 12.67% in Klebsiella species, isolated from urinary tract infection[11] and Sarumathi D et al.[8] reported 20.4% colistin resistance in CRE isolates.[8] Also, to note few studies have reported extremely high resistance to colistin. Qadi M, et al.[3] has reported 41% of Enterobacterales isolates were colistin resistant[3]; L. Bardet et al.[9] reported 63.4% of colistin resistance among gram negative bacilli,[9] and also, Capone A, et al.[11] reported 36.1% of carbapenem resistant *K. pneumoniae* were colistin resistant.[11]

CBDE and CAT was performed, and it was compared with reference BMD. Categorical agreement was analysed based on EUCAST 2021 guidelines. Essential agreement was not analysed

as CBDE and CAT was performed only in 3 dilutions, and BMD was performed in 8 dilutions.

As shown in Table 4, 2 isolates showed MIC of 2 in CBDE method and MIC of 4 in reference method, (very major error) in CBDE with respect to reference method and CA of CBDE was 98% with reference BMD. Similar findings were observed by Humphries RM et al.[1] in which they reported 97.9% categorial agreement compared to the reference MIC and reported 9 VME (3.2%).[1] In a 2 site evaluation of CBDE, conducted by Simner PJ, et al.[12] they have concluded that CBDE test had 100% CA with reference method in both the site evaluation centres.[12] In a study conducted in two different research centres in Brazil, Dalmolin TV et al., reported 91.18% of CA and also noted 4.95% of VME with CBDE compared to reference method.[13]

As shown in Table 5, 1 isolate showed MIC of 2 in CAT method and MIC of 8 in reference method (very major error) and CA of CAT was 99% with reference BMD. Similar to the present study, Humphries RM et al.[1] reported CA of 98.3% with 3.9% VME for CAT.[1] Also, to note, Lellouche J, et al., reported[14] CA of 97.3% and VME of 10.2% for CAT.[14]

With respect to performing CBDE and CAT, it was found that CBDE requires large volume MHB (every isolate requires 40ml of MHB; 4 number-10ml test tubes). It becomes very tedious to arrange large volume MHB and test tubes while performing CBDE on routine basis and this may not be economically feasible. CAT is comparatively easier to perform as every dilution plate of CAT can be inoculated up to 10 isolates. Another noteworthy point while performing CBDE is that the colistin disk to be used in the test should be of high standards and should contain appropriate potency to ensure proper elution of the disk. Most of the literature on CBDE by western world has utilized high end company colistin disk, whereas in Indian settings it may become questionable to procure high standard disk for routinely performing colistin AST. Hence, authors would like to recommend use of CAT method to routinely report colistin MIC to the clinicians.

CONCLUSION

With the increasing colistin resistance, it should be a mandate scope of every Microbiology laboratory to report colistin MIC by validated method, so that clinicians will use this high-end drug with caution. We hereby conclude that CAT test is feasible and can be implemented as a part of routine antimicrobial susceptibility testing of colistin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analysed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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