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Research Article

COLISTIN SUSCEPTIBILITY TESTING OF CARBAPENEM RESISTANT ENTEROBACTERIACEAE- A COMPARATIVE STUDY OF BROTH MICRODILUTION METHOD AND VITEK 2 METHOD

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Abstract- Comparative study conducted in the Department of Microbiology in a tertiary care teaching hospital during December 2019 to May 2021. Minimum inhibitory concentration of colistin determined by broth microdilution method for clinical isolates of Carbapenem resistant Enterobacteriaceae (CRE)and compared with the results obtained in Vitek 2 Compact. A total of 205 CRE isolates were included in the study. *Klebsiella pneumoniae* (60.5%) was the predominant CRE isolate, followed by *E. coli* (36.6%) and *Enterobacter cloacae* (2.9%). MIC ranging from 0. 0625 µg/ml to 0.5 µg/ml were obtained for our study isolates. All the 205 Carbapenem resistant Enterobacteriaceae isolates in the study were found to be susceptible to colistin both by BMD method and VITEK 2 method. There was 100% categorical agreement between microbroth dilution method and VITEK 2 system for colistin susceptibility. Our study results showed that automated method, VITEK 2 compact system can be used as reliable alternative method for reporting colistin susceptibility.

Keywords- *Carbapenem resistant Enterobacteriaceae, Colistin susceptibility testing, Broth microdilution method, Vitek 2 method*

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Introduction

Enterobacteriaceae family is an important cause of urinary tract infections, bloodstream infections, hospital acquired pneumonias and various intra-abdominal infections. Incidence of infections with Carbapenem resistant Enterobacteriaceae has increased over the past few years. The polymyxins (colistin and polymyxin B) are antibiotics which are currently used for treating infections with CRE [1,2]. An accurate method for antimicrobial susceptibility testing of colistin is crucial in this era of increasing numbers of multidrug-resistant Gram-negative bacteria and simultaneous increasing colistin resistance. Even though, Micro broth dilution method is the gold standard reference method for antibiotic susceptibility testing of colistin, clinical microbiology laboratories only rarely perform this method for routine susceptibility reporting. In this study we have performed broth microdilution (BMD) method for colistin susceptibility testing and compared it with that of routinely performed Vitek 2 susceptibility results.

Materials and Methods

A study was conducted in Department of Microbiology in a tertiary care teaching hospital in Kerala. Study included Enteriobacteriales obtained from pus, sputum, blood and urine samples. Organisms from Enterobacteriaceae family showing intrinsic resistance to colistin and repeated isolates from the same patient were excluded. Identification of all isolates to species level and antibiotic sensitivity were done by VITEK 2 Compact (Biomerieux) system. Vitek 2 AST N280 susceptibility card was used to perform the antibiotic susceptibility testing. Isolates having Meropenem MIC >=16 µg/ mL, is taken as carbapenem resistant isolate [3]. Colistin susceptibility of carbapenem resistant isolates were further determined using broth micro dilution method. Broth microdilution was performed according to joint CLSI-EUCAST recommended guidelines and ISO 20776:2006 [4-6]. Antibiotic stock solution was prepared using cation adjusted Mueller-Hinton broth (HI media M1657) following manufacturer's instructions.

The stock solution of colistin was prepared from colistin sulphate salt (Sigma C4461). A range of 2-fold dilutions of colistin concentrations (ranging from 0.0625 to 16 µg/ml), and final bacterial inoculum size of 5×10^5 CFU/mL were used. The test was done in untreated sterile polystyrene microtiter plate (Tarsons 96 well micro test plate U bottom wells) and incubated for 16 to 20 hours at 37°C and examined visually by two observers and MIC values were noted. For quality control, ATCC 25922 *Escherichia coli* was used. For sterility control, lowest concentration antibiotic solution without bacterial inoculum was added to wells. To the growth control well 100 µl of inoculum without antibiotic was added. Growth control and sterility controls were checked before reading every results. Lowest concentration of colistin at which no visible growth obtained was recorded as MIC.
Skip well phenomenon was defined as the absence of growth of an isolate at lower antimicrobial concentration(s).
A single skip well did not affect the MIC interpretation, while multiple skip wells were considered as uninterpretable according to CLSI guidelines.4 In case of single skipped well highest concentration was taken as MIC and for multiple skipped wells retesting was done. Because the CLSI does not provide clinical breakpoints for colistin for Enterobacteriaceae, European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC breakpoints was used for interpretation (MIC ≤ 2 µg/ml – susceptible, MIC >2 µg/ml – Resistant). Performance of Vitek 2 to determine colistin susceptibility were evaluated by comparing with that of BMD.
Any errors in colistin susceptibility result were noted and categorical agreement (CA) was calculated (Percentage of isolates in the same susceptibility category by BMD and the method under evaluation). Performance errors were categorized as follows; very major errors (false susceptible results), major errors (false resistant result) and minor errors (related to an intermediate interpretation for either the reference or test method) [7].

Results

Total of 205 nonrepetitive CRE isolates were included in the study. *Klebsiella pneumoniae* was the predominant CRE isolate in our study, followed by *E. coli* and Enterobacter cloacae [Fig-1]. All the isolates included in the study were obtained from patients admitted in either ward (69.7%) or ICUs (30.3%). Majority of CRE isolates were obtained from urine followed by blood, sputum and pus aspirates or exudates [Table-1]. Out of the 205 CRE isolates, 99 % isolates were susceptible to Tigecycline and 18% were susceptible to aminoglycosides. Out of the total 110 urinary isolates, 80% showed susceptibility to Nitrofurantoin [Table-2].

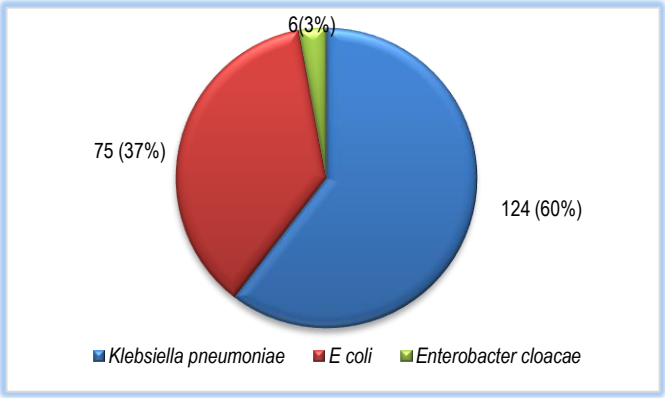

Fig-1 Frequency distribution of CRE clinical isolates n(%)

Table-1 Sample wise distribution of CRE isolates n(%)

Organism(n)	Urine	Blood	Sputum	Pus
Klebsiella pneumoniae (124)	63 (50.8%)	23 (18.5%)	22 (17.7%)	16 (12.9%)
E. coli (75)	42 (56%)	12 (16%)	14(18.6%)	7 (9.3%)
E. cloacae (6)	5 (83.3%)	0	1 (16.6%)	0
Total- 205	110 (53.6%)	35 (17%)	37 (18%)	23(11.2%)

Highest and lowest colistin MIC obtained for study isolates was 0.5 µg/ml and 0.0625 µg/ml respectively by BMD method. Majority of our isolates had MIC value of 0.625 µg/ml and only five isolates have shown MIC value 0.5 µg/ml, which included four isolates of *Klebsiella pneumoniae* and one isolate of *E. coli* [Table-3]. All the isolates showed colistin MIC of < 0.5 µg/ml by Vitek method.

According to the colistin susceptibility interpretation there was 100% categorical agreement between Vitek and standard reference BMD method. Essential agreement could not be determined as in Vitek 2 system colistin concentrations lower than 0.5 µg/ml are not tested.

Discussion

The emerging resistance in Gram negative bacilli poses necessity to find a reliable susceptibility testing method for last line drugs like colistin. Polymyxins are large cationic peptide molecules and do not diffuse through agar well, hence disk diffusion is neither reliable nor recommended. Various studies have compared different susceptibility testing method against the reference broth microdilution method. In the present study we tried to find out the colistin susceptibility among CRE isolates using microbroth dilution method in clinical isolates and also compared with that of Vitek 2 method.

In this study, the most common CRE isolate obtained was *Klebsiella pneumoniae* (60.5%) followed by *E. coli* (36.6%) and Enterobacter cloacae (2.9%). Many studies worldwide including those from India, have reported *Klebsiella* as the most common CRE organism in their studies [8-12]. In our study, majority of CRE isolates were obtained from urine (53.7%) followed by blood (18%), sputum (17.1%) and pus aspirates or exudates (11.2%). Similarly previous studies from India have also reported urine and respiratory specimens as the common source of CRE isolates [12,13]. All our study isolates were obtained from inpatients, in contrast to study by Ayushi Sharma *et al*, where they had isolates from our patients as well.

In our study all isolates were colistin susceptible and majority of our study isolates had lower colistin MIC of 0.0625 µg/ml. Other studies from different parts of India have shown varying number of colistin resistance among CRE isolates [14-16]. These studies have reported higher of colistin MIC especially for carbapenem resistant *Klebsiella pneumoniae*. Our institute have well-functioning hospital infection control team and also have implemented antibiotic stewardship program. Major part of our study was conducted during COVID pandemic so there a was marked drop in IP admissions during this period. These factors can well explain the reasons for lower colistin MIC for our study isolates.

Table-2 *Antibiotic sensitivity profile of CRE isolates*

Antibiotics	No. of Susceptible isolates (%)
Tigecycline	203 (99%)
Cotrimoxazole	107(52.2%)
Amikacin	37(18%)
Gentamicin	37(18%)
Ciprofloxacin	0(0%)
Nitrofurantoin(n=110)	88(80%)

A false susceptible result is obviously a very major error but in a last resort agent like colistin, a false resistant result is just as unfortunate and should be considered equally serious. Hence it is absolutely essential for laboratories to report correct colistin susceptibility results at the earliest. Even though BMD is the standard reference method for colistin susceptibility testing it is not done routinely due to the cumbersome procedure. So, most of the laboratories depend either on automated methods or commercial broth dilution method for the same purpose. In our study there was 100% categorical agreement between Vitek and standard reference BMD method for colistin susceptibility. However, it is not possible to comment on the exact CA between these two methods as there were no colistin resistant isolates both by BMD and Vitek method in our study. Previous studies reported CA varying from 90 to 96% between BMD and Vitek 2 method. Studies have reported varying degrees of CA among different organisms as well [17-19]. Das S et al reported CA between 75% and 96% among Enterobacteriaceae and lowest CA of 75% was reported for Enterobacter species in their study. Essential agreement between these two methods also could not be analyzed in our study.

Conclusion

Microbroth dilution, the currently recommended gold standard method for colistin susceptibility testing is a laborious and difficult to perform on daily basis. Our study results showed that automated method, VITEK 2 compact system can be used as reliable alternative method for reporting colistin susceptibility.

Application of research: Evaluation of automated method for performing colistin susceptibility testing in routine clinical Microbiology laboratories.

Research Category: Clinical Microbiology

Abbreviations: CRE- Carbapenem resistant Enterobacteriaceae
BMD- Broth microdilution method
MIC- Minimum inhibitory concentration, CA- Categorical agreement

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Research project name or number: Research station study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Tertiary care teaching hospital in Kerala

Table-3 *Distribution of colistin MIC among the CRE isolates*

Organism (n)	Method	MIC (µg/ml)		No: of isolates with MIC(µg/ml) (%)			
		MIC_{50}	MIC_{90}	0.0625	0.125	0.25	0.5
Klebsiella pneumoniae (124)	BMD	0.125	0.25	57(46%)	43(34.8%)	20(16.1%)	4(1.9%)
E. coli (75)	BMD	0.125	0.25	33(44%)	28(37.3%)	13(17.3%)	1(0.5%)
Enterobacter cloacae (6)	BMD	0.0625	0.125	4(66.7%)	1(16.7%)	1(16.7%)	0

Strain name: *Klebsiella pneumoniae*

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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