

Comparison of disc diffusion antibiotic sensitivity pattern and Minimum Inhibitory Concentration for meropenem resistant coliform isolates: can increasing the dosage give more coverage?

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ABSTRACT

Objective

Patients with hematological malignancies are vulnerable to Carbapenem Resistant Enterobacteriaceae (CRE) infections. Meropenem is a broad-spectrum antibiotic used to treat Enterobacteriaceae infections. Determination of minimum inhibition concentration (MIC) using broth microdilution (BMD) is the gold standard in defining optimal treatment. The objective was to compare the disc diffusion antibiotic sensitivity pattern and MIC for the meropenem resistant coliform isolates at National Cancer Institute Maharagama, Sri Lanka.

Materials and methods

A total of 977 clinical isolates were collected and susceptibility to meropenem was assessed by the disc diffusion (DD) method. Out of that, 87 resistant coliform isolates were randomly selected and MICs were tested using the BMD method. For the blood culture isolates (n=32), results were also obtained using VITEK2 automated system and were compared with BMD and DD results.

Results

Out of 494/977 (50.6%) meropenem resistant isolates, majority were resistant to coliforms (67.2%). A total of 87 meropenem resistant coliform isolates by DD method were further analyzed, out of which 85.1% (n=74) were resistant, 13.8% (n=12) were intermediate and 1.19% (n=1) were sensitive by the BMD method. No significance difference between the DD method and the BMD method was observed (P>0.05). Out of 32 blood culture isolates that were resistant by the DD method, one was sensitive and one was intermediate by VITEK2 method. Error analysis of blood culture isolates showed very major errors between BMD and VITEK2 (3.13%) in one sample while frequent major errors in DD and VITEK2 compared with the BMD, which ranged from 3.13% to 18.75% respectively.

Conclusion

Increasing the dose based on the MICs will result in the susceptibility in few of the isolates which were previously detected as resistant by the DD method. It can be further hypothesized that for the isolates with MICs above the therapeutic dose, alternative strategies such as combination therapy of meropenem and the development of novel antibiotics, are needed for the effective management of meropenem resistant strains.

Keywords

Broth micro dilution; coliforms; Enterobacteriaceae; meropenem resistance; MIC.

INTRODUCTION

Antibiotics are among the most commonly used medications in modern medicine. Though, around half of the prescriptions are considered unnecessary.¹ Antimicrobial resistance (AMR) is increasing across the world and has become very challenging to control as a result of rapid development of Multi Drug Resistant (MDR) bacteria and lack of reliable surveillance techniques.² Although the emergence of AMR in micro-organisms is a natural occurrence, the speed of resistance has increased by the excessive and inappropriate use of antimicrobial drugs.^{3,4} Since many new antimicrobial medicines are not available on the market to replace those that have proved ineffective, their efficacy must be protected.

Carbapenems are broad spectrum, beta-lactam antibiotics that are used for treating life-threatening serious infections which do not respond to standard antibiotic therapy and has the ability to kill micro-organisms based on concentration-independent killing.^{5,6} Carbapenems are effective in treating infections caused by wide range of bacteria. They are

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considered as one of the last remaining antibacterial alternatives for drug resistant Gram negative bacteria (GNB).^{7,8} Meropenem, imipenem, ertapenem, and doripenem are the most clinically used carbapenems.⁹ According to the Ambler classification system, Carbapenem Resistant *Enterobacteriaceae* (CRE) are classified into 3 groups as class A, class B, and class D.¹⁰ CRE such as *Klebsiella pneumoniae* and *Escherichia coli*, are a major threat due to the acquisition of carbapenemase genes. These genes encode specialized beta-lactamase enzymes, that inactivate carbapenem antibiotics by hydrolyzing the beta-lactam ring of the drug. The rapid global spread of carbapenem resistance is facilitated by these genes being located on mobile genetic elements, allowing for horizontal gene transfer.¹¹ Several studies have reported a recent worldwide rise of carbapenem resistance among ESBL producing *Klebsiella pneumoniae* and *E. coli* isolates.^{12,13}

Patients with hematological malignancies are frequently neutropenic and require prolonged antibiotic courses, making them highly vulnerable to infections caused by MDR GNB. This has resulted in the alarming emergence of CRE, with meropenem resistance being particularly prominent.¹⁴⁻¹⁷

Disc diffusion (DD) method, broth microdilution (BMD) method, and rapid automated method are the commonly used test methods for detection of antimicrobial resistance. The Minimum Inhibitory Concentration (MIC) detection, which gives a quantitative result is considered as the gold standard method for antimicrobial susceptibility testing.^{18,19} The institutions of EUCAST and CLSI both recommend BMD method as the preferred technique for MIC testing.²⁰⁻²⁴ In Sri Lanka, DD method is used to test the susceptibility. If an antibiotic shows resistance by the disc diffusion method, that drug cannot be administered any longer in the same dose. However, if the MICs are known the drug can be further used by increasing the dose up to the therapeutic dose. Thus, it's worthwhile finding the possibility of using a higher dose than the susceptible breakpoint dose.²⁰ The current study aimed to compare disc diffusion antibiotic sensitivity pattern and MIC for meropenem resistant coliforms isolated from National Cancer Institute Maharagama (NCIM) Sri Lanka.

MATERIALS AND METHODS

Meropenem resistant coliform isolates received to the microbiology laboratory of NCIM, Sri Lanka from

September 2020 to January 2021 were selected as the study sample. Ethical clearance was obtained from the ethics review committee of Kotelawala Defence University (KDU) (RP/S/2020/17). Data were collected from existed clinical and routine laboratory records. No direct patient contact was occurred, and no new biological samples were collected specifically for this study. The collected data from the laboratory was devoid from any patient identification data. The project was entirely carried out from the leftover specimens received to the clinical microbiology laboratory. All the coliforms isolated from urine, pus, sputum, and blood specimens were tested for meropenem-resistance by the DD method and results interpreted as per CLSI guidelines.²⁰

Both BMD method and VITEK2 automated method were used to analyze the MIC values. The MICs of all the selected 87 isolates were determined by the BMD, while only for blood culture isolates (n=32), MIC values were analyzed by VITEK2 automated method also due to the limitation of resources. In BMD method, a working solution of meropenem with the concentration of 64µg/ml was prepared to maintain the QC range (0.008-0.06µg/ml) and not to exceed the therapeutic dose range (≤16µg/ml). Serial two-fold dilutions were prepared by diluting 64µg/ml meropenem working solution in 96 microwell plate with 100µl of Mueller Hinton Broth and a final meropenem concentration of 16µg/ml was obtained. The microwell plates were incubated at 35°C for 16 to 20 hours covering with a tight-fitting plastic cover. The MICs were interpreted as sensitive (≤1µg/ml), intermediate (2µg/ml), or resistant (≥ 4µg/ml) following the manufacturer's instructions.

All clinical data collected from laboratory records and results from laboratory investigations were analyzed and interpreted using SPSS version 20 and GraphPad Prism 9.3.1. Variables were classified and descriptively analyzed. Errors were ranked compared to the reference, BMD method as follows. Very major errors (VMEs), false susceptible results by DD and/or VITEK2 methods; major errors (MEs), false resistance by DD and/or VITEK2 method and minor errors (MiEs), intermediate result by DD and/or VITEK2 method and sensitive or resistant by BMD method. Unacceptable levels according to CLSI M23-A2 document are, ≥ 1.5% for very major error, ≥ 3% for major error and 10% for minor error²⁴. Statistical significance was considered as p< 0.05.

RESULTS

A total of 977 coliforms were isolated during the study period. Of which, meropenem-resistance was detected among 50.6% (n-494/977). The Table 1 represents descriptive statistics of the study population. Out of 494 meropenem resistant coliform isolates, only 87 were selected to perform MICs due to the limitation of laboratory access during the prevailed Covid-19 pandemic situation. The meropenem zone diameters of selected 87 coliform isolates were below 19mm and hence considered as resistant isolates. Meropenem resistance was most commonly observed among blood culture isolates (36.8%) as shown in Table 2.

Table 1. Meropenem susceptibility according to organism type in the study population

Organism	Meropenem susceptibility				Total
	Resistant		Sensitive		
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
Coliforms	332	67.2	347	71.8	679
<i>Pseudomonas</i> spp.	74	15.0	103	21.4	177
<i>Acinetobacter</i> spp.	88	17.8	33	6.8	121
Total	494	100.0	483	100.0	977

Table 2: Distribution of meropenem resistant isolates selected for MIC assay (n=87)

Type of specimen	Frequency	Percentage
Blood	32	36.8%
Urine	30	34.5%
Sputum	17	19.5%
Pus	8	9.2%

Out of the 87 isolates, the highest frequency was LF coliforms (77%, n=67), while only 23% (n=20) were

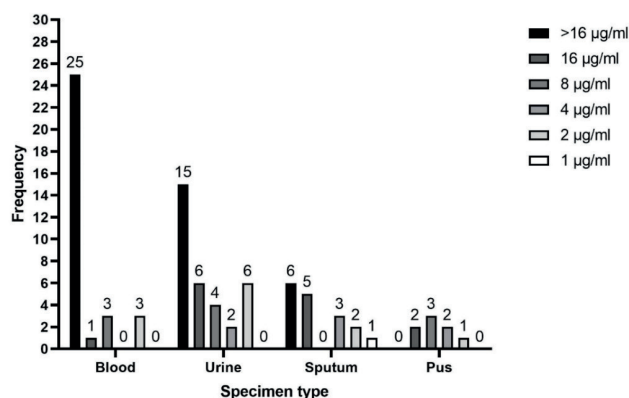


Figure 1. Distribution of MIC values obtained by BMD method, among the isolates collected from different sample types

NLF coliforms. Among the isolates which categorized as meropenem resistant by the BMD method, a majority (49.43%) showed >16 µg/ml as the MIC value with blood culture isolates showing the highest frequency as shown in figure 1.

Meropenem susceptibility patterns of isolates by BMD and DD methods are shown in table 3. Accordingly, 74 isolates showed meropenem resistance by both DD and BMD methods (Table 3). No significance difference was noted between DD method and the BMD method ($P>0.05$) in the sample.

Table 3. Meropenem susceptibility by DD method and BMD method in the sample

Susceptibility testing method (n=87)	Sensitive	Intermediate	Resistant
By DD method	0	0	87 (100.0%)
By BMD method	1 (1.1%)	12 (13.8%)	74 (85.0%)

Out of 32 blood culture isolates that were resistant by the DD method initially, one was sensitive and one was intermediate sensitive by VITEK2 automated method (Figure 2). The error analysis results are represented in Table 4. However, DD method showed a greater frequency of MEs compared to BMD method as six samples did not match.

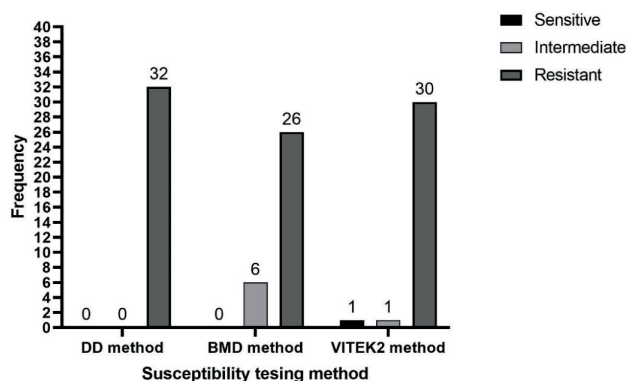


Figure 2. Comparison of meropenem susceptibility of blood culture isolates (n=32) which were analyzed by all the three methods (DD, BMD and VITEK2)

Table 4. Error rates of DD and VITEK2 methods compared to BMD (reference) method for 32 blood culture isolates

Antimicrobial susceptibility testing method	Number of discrepancies (%)		
	No. of very major errors (VMEs)	No. of major errors (MEs)	No. of minor errors (MiEs)
BMD and DD	0	6 (18.75%)	0
BMD and VITEK2	1 (3.13%)	1 (3.13%)	0

DISCUSSION

CRE is an emerging health concern. Patients having hematological malignancies are neutropenic and vulnerable to CRE infections as they rely on immediate antibiotic treatment against Gram negative infections. Infections due to CRE are the most prominent cause of Gram-negative bacteremia, where meropenem is a drug of choice among this population. The BMD method is considered as the gold standard for determining the optimal treatment and recognizing AMR. Epidemiological statistics of carbapenem resistance are comparatively less noted in Sri Lankan clinical setting.²⁵ The main objective of this study was to compare the disc diffusion antibiotic sensitivity pattern and MIC for the meropenem resistant coliforms isolated from the NCIM, Sri Lanka.

In the present study, overall meropenem resistance rate was 50.6% (n=494) and it was higher than the number of meropenem sensitive (n=483) isolates. A study done in Sri Lanka from 2015 to 2016 reported 11% prevalence of carbapenemase producers while, it remained around 9.5% in 2017-2018, and has shown a slow increase of carbapenem resistance in Sri Lanka over the years.^{26, 27} Increased carbapenem resistant rates have been reported globally as well, which ranged from 0.08% to 80% indicating a massive difference between developing and developed countries.^{28,29} The lack of public health surveillance programs in majority of countries, could be a reason for this emergence and rapid growth of AMR. These findings highlight the urgent need to address antibiotic resistance in healthcare settings.

Carbapenem resistance may vary depending on the type of organism. Both fermenters and non-fermenters are responsible for carbapenem resistance. According to the present study findings, the highest occurrence of carbapenem resistance (67.2%, n=332) was observed in coliforms. Several published articles also reported carbapenem resistant coliform isolates as the most common organism supporting the findings of our study.^{30,31} However, different results were reported in various countries indicating dynamic variations within countries.³²⁻³⁵ Carbapenem resistance rates may also vary depending on the site of infection.³² In this study, the highest carbapenem resistance was observed in urine culture isolates (31.93%). In contrast, the highest CRE prevalence was noted in sputum samples as reported from Sri Lanka and elsewhere.^{36,37} Out of 32 blood culture samples, majority (65.62%) of the resistant organisms were *K. pneumoniae* with a MIC of >16µg/ml which is in line with another published article.³⁸ This is concerning, as coliforms are responsible for a large variety of infections, including UTIs and bloodstream infections. The high resistance rate observed in coliforms emphasizes the increasing challenge in managing these infections effectively. Furthermore, the type of specimen from which the resistant isolates were obtained, provide a valuable information regarding the clinical relevance and potential sources of these bacterial strains.

Notably, all 87 selected isolates exhibited zone diameters below 19mm, indicating the meropenem resistance, based on CLSI interpretation criteria. In Sri Lankan hospital setting, meropenem susceptibility is determined according to the zone diameter values,

as the use of zone diameter measurements is a reliable initial indicator of meropenem resistance. Majority (49.43%) of the meropenem-resistant isolates showed MIC values above 16 µg/ml by the BMD method. Elevated MIC values suggest a high resistance level, posing a significant clinical concern. Moreover, the occurrence of high MIC values in blood culture isolates emphasizes the severity of meropenem resistance in bloodstream infections. These infections often require prompt and effective treatment.

Discrepancies were observed when comparing the DD and the BMD resistant results. Although, the DD method classified all the selected 87 isolates as resistant, the BMD method identified 85.05% (n=74) as resistant, 13.8% (n=12) as intermediate sensitive, and only 1.1% (n=1) as sensitive. However, a statistically significant difference was unable to observe between the DD method and the BMD method ($P>0.05$). When the error rates were compared between VITEK2 and BMD methods, only one (3.13%) VME was observed in the VITEK2 method and frequent MEs (21.88%) were observed in both DD and VITEK2 methods, in comparison with the BMD method. However, these findings are in accordance with the published studies in Brazil,²⁴ Tunisia,³⁹ Singapore,⁴⁰ and USA,⁴¹ where high error rates were also reported. VMEs are concerning as it can lead to the inappropriate prescribing of antibiotics for diagnosed microbes. MEs should also be concerned, as it may cause failure of treatments and finally increase the spread of AMR. MiEs are also important, as these may cause unnecessary changing of treatment plans, increment of health sector related cost and delaying of antibiotic treatment to patients. Therefore, it's important to confirm the meropenem resistance using more sensitive methods such as, MIC detection by BMD method. This discrepancy highlights the importance of using a standardized and validated method such as the BMD method for antimicrobial susceptibility testing, to ensure the accuracy and reliability of the results. The variation in interpretation emphasizes the need for further evaluation and the establishment of consistent guidelines for interpretation of meropenem resistance.

These findings suggest the importance of exploring alternative treatment options such as, combination therapy or alternative antibiotics with different mechanisms of action. Combination therapy, where meropenem is used in conjunction with other antibiotics

may enhance efficacy of meropenem and overcome resistance mechanisms. Additionally, the development of new antimicrobial agents targeting meropenem-resistant strains could provide alternative treatment options in the future.^{42,43} Though meropenem is resistant by DD method, if the MIC values are known, it can be administered by increasing the dose up to the therapeutic dose. The rationale behind the increasing of drug concentration lies in the assumption that higher meropenem concentrations may overcome bacterial resistance mechanisms. This will lead to improve bacterial clearance and treatment efficacy. However, immense care should be given on the therapeutic dose.

There were few limitations in the present study. This study was performed in a single center, comparatively over a short period of time. In addition, due to the prevailed pandemic situation, access to the laboratory was limited. As a result, the study was conducted with a relatively small sample count, limiting the generalizability of findings to a large population. Further studies need to be performed in multiple centers with a large sample size. To determine a more precise dosing regimen, it would be better to consider MIC values in addition to results given by DD method.

CONCLUSION

High occurrence of meropenem resistant coliforms were observed among the patients attending to the NCIM. Analysis of meropenem resistance by DD and BMD methods provided comparable results. There were no statistically significant differences between the results obtained by the DD method and the BMD method. However, overall error analysis of blood culture isolates represented frequent MEs and one VME indicating discrepancy of results obtained by different methods. Therefore, it's important to employ accurate and reliable methods to assess AMR patterns, considering the limitations and discrepancies among conventional methods. Increasing the meropenem concentration up to the therapeutic dose, is a potential strategy to address the resistance. The study findings indicate that, this approach alone may not be sufficient. Alternative treatment strategies, such as combination therapy of meropenem and the development of novel antimicrobial agents, are required to effectively manage infections caused by meropenem-resistant strains.

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Authors contribution statement

Conceptualization- TS, VN, SG

Data curation- NJ, CW, CP, RC, NK

Formal analysis- NJ, CW, CP, RC, NK

Investigation- NJ, CW, CP, RC, NK

Methodology- NJ, CW, CP, RC, NK

Project administration- TS, VN, SG

Resources- CW, CP, RC, NK

Supervision- TS, VN, SG

Visualization- NJ, CW, CP, RC, NK

Writing-original draft preparation- NJ

Writing-review and editing- NJ, TS, VN, SG

All authors read and approved the final version of the manuscript.

Conflicts of interest- All authors-none to declare

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