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Comparison of Automated and Manual Methods for Antimicrobial Susceptibility Testing

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Authors' contributions

This work was carried out in collaboration among all authors. Author AB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors DA and SN performed the statistical analysis and literature searches. Author AB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Carbapenems are considered the broadest-spectrum β-lactam agents and are often required for treatment of severe hospital-acquired infections caused by multidrug-resistant Gram-negative organisms. Minimum inhibitory concentrations (MICs) are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.
Aims and Objectives: To compare the MIC obtained by Broth Microdilution method (BMD) with that of Vitek-2(automated method) for recovered isolates of *Klebsiella pneumoniae*.
Materials and Methods: Prospective study conducted over a period of one year. It included all isolates of *Klebsiella pneumoniae* recovered from blood culture of the patients. The identification and antimicrobial susceptibility was done on Vitek-2.These Isolates were subjected to Microbroth dilution method for MIC determination.
Results: Out of the 55 meropenem resistant isolates by vitek-2, 20(36.3%) had MIC of ≥256 µg/ml followed by 18(32.7%) isolates with a MIC of 128 µg/ml, followed by 11(20%) isolates with MIC of 64 µg/ml and 6(10.9%) isolates with MIC of 32 µg/ml. Also among 15 meropenem sensitive isolates by Vitek-2, 13(86.7%) had MIC of ≤0.5 µg/ml, followed by two (13.3%) isolates with MIC of 2 µg/ml.

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Results obtained by vitek 2 were compared with those from BMD(the reference method), which showed a 13.3% minor error rate and no major or very major error rate.
Conclusion: Overall, the Vitek 2 performance was comparable to that of BMD for testing a limited number of *Klebsiella pneumoniae* isolates.

Keywords: Klebsiella pneumoniae; minimum inhibitory concentration; Vitek-2; MBD.

1. INTRODUCTION

Carbapenems are considered first-line therapy for infection with multidrug-resistant *Enterobacteriaceae*. [1] Carbapenemase - producing gram negative bacteria result in serious infections leading to an extension of the period of hospitalization and increase in the mortality ratio.The increasing emergence of serine-based carbapenemase-producing *Klebsiella pneumonia* (KPC) worldwide is of growing concern [2]. Therefore, monitoring of development of resistance against carbapenems is necessary [3].

*Klebsiella pneumoniae*is one of the most important gram negative bacterial pathogen which has caused worldwide concern because of its association with life threatening nosocomial infections and its multidrug resistant (MDR) property. Owing to its ability to produce extended spectrum-β-lactamases (ESBL), carbapenems have become the preferred antimicrobial for treating such conditions which in turn has resulted in emergence of the strains which are carbapenem resistant [4].

Most clinical laboratories use commercial automated systems for antimicrobial susceptibility testing (AST). The failure of these systems to detect resistance in *Enterobacteriaceae*, in particular the β-lactam resistance mediated by emerging resistance mechanisms, has been reported in several studies [5-7]. Utilization of reliable methods for identifying carbapenemase-producing strains and determining their antibiotic resistance pattern could have a very important role in treatment of infections caused by these strains, which could be an important step in the control of hospital in-fections, in order to prevent patients' mortality and to reduce health care costs [8,9].

The most commonly used method for detection of CRE is the measurement of minimum inhibitory concentration (MIC). MICs are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobialagent [10,11]. Thus objective of this study was to compare meropenem MIC and susceptibility testing for *Klebsiella pneumoniae* by Vitek-2 and Broth Microdilution Method. We considered the BMD to be the reference method and tested this automated system against this standard.

2. MATERIALS AND METHODS

This was a prospective study conducted in the Department of Microbiology at Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Soura, Srinagar, Kashmir, a 700 bedded tertiary care hospital. Blood culture bottles that flagged positive were subcultured on Blood agar and MacConkey agar to be incubated at 37°C overnight. Theinocula prepared were processed byVitek-2 system (with software release 2.01) and by Broth Microdilution method for comparison of MIC.

2.1 MIC by Broth Microdilution Method as Under

2.1.1 Preparation of antibiotic stock solution for meropenem

Stock solution was prepared using the formula

$$1000/P \times V \times C = W$$

Where, P= potency given by manufacturer (µg/mg), V = volume required (ml), C = final concentration of solution (mg/ L) and W = weight of antibiotic (mg) to be dissolved in volume V (ml). The stock solution was prepared in such a way that its concentration was 1mg/ml or greater. Meropenem stock solution was prepared by dissolving 55.43 mg of the antibiotic powder in 1ml of distilled water.

2.1.2 Preparation of working antibiotic solution

Working solution was prepared as per the formula V1C1= V2C2 (V1=volume of starting solution needed, C1-concentration of starting solution needed, C2=final concentration of new solution, V2=final volume of new solution). The working solution was prepared one concentration higher than the highest concentration of the drug being tested. Thus for meropenem, 256 µg/ml of

working solution was prepared by dissolving 51.2µl of stock solution in Muller-Hinton broth.

2.1.3 Broth microdilution method

Using a micropipette 50 µl of Muller Hinton broth was dispensed into all wells of a microtitre plate leaving the first column unfilled. After this 100µl of working antibiotic solution (concentration 256 µg/ml) was added to the wells of the first column. Fromthe first well 50 µl of the working antibiotic solution was pipetted out and added to the second well, already containing 50 µl of MH broth .From the second well 50 µl of solution was added into the next well and so on and so forth till the well well number 10 was reached from which 50 µl of solution was discarded. The final concentration in the wells ranged from 256-0.5 µg/ml. The last two columns served as growth control and sterility control respectively. The turbidity of the bacterial inoculum was adjusted to 0.5 McFarland standards and 50 µl of it was dispensed into all the wells of microtitreplate. Finally the plates were incubated at 37ºC overnight and read the other day.

Results were recorded by visual inspection of the microtitre plates after overnight incubation at 37ºC as per CLSI guidelines. The test was considered valid when acceptable growth (more or equal to 2 mm button or definite turbidity) was seen in the positive control well. Absence of turbidity or a button of less than 2 mm diameter in the test well was thus taken as the MIC of the organism under test [12].

3. RESULTS

In our study a total of 70 non duplicate *Klebsiella pneumoniae* were isolated from patients admitted or attending the OPD. Out of the total isolates 55 (78.5%) were meropenem resistant and 15 (21.5%) were meropenem sensitive by Vitek-2.Minimum Inhibitory Concentration (MIC) was done on these isolates by Broth microdilution test. For (36.3%) isolates MIC was ≥256 µg/ml followed by 128 µg/ml in (32.7%) isolates followed by 64 in (20%) isolates and 32 in (10.9%) as shown in Table 1.

Table 1. MIC of meropenem for *Klebsiella pneumoniae* isolates by Vitek-2 and BMD

Concentration of antibiotics	VTK	BMD
MIC <=0.5 mcg/ml	15	13
MIC: 1 mcg/ml	-	-
MIC: 2 mcg/ml	-	2
MIC: 4 mcg/ml	-	-
MIC >16 mcg/ml	55	55
Total No. of Isolates		70

4. DISCUSSION

Carbapenem resistance among Entero bacteriaceae members is of great concern as these bacteria are easily transmissible among patients, leading to hospital acquired infections (HAI), but can also spread into the community, resulting in community acquired cases [13].

There is a need to provide rapid, efficient and accurate system for identification and antimicrobial susceptibility testing of these pathogens. In this regard the automated identification/AST systems aid in rapid diagnosis/treatment of bacterial pathogens [14].

The objective of this studywas to compare meropenem susceptibility testing for *Klebsiella pneumoniae* by BMD and Vitek-2 .We considered the broth microdilution method to be the reference method and tested automated systems (Vitek 2) against this standard.

In our study a total of 70 non duplicate *Klebsiella pneumoniae* were isolated from patients admitted or attending the OPD. Out of

Table 2. Interpretive results for *Klebsiella pneumoniae* isolates

Testing method	No. (%) of isolates		
	Susceptible	Intermediate	Resistant
BMD	13	2	55
VTK	15	0	55

Table 3. Comparison of MIC of meropenem for *Klebsiella pneumoniae* by Vitek-2 and BMD

Isolates resistant by Vitek-2		Isolates sensitive by Vitek-2	
MIC	Total no. (%)	MIC	Total no. (%)
32 mcg/ml	6(10.9)	0.25 mcg/ml	5(33.3)
64 mcg/ml	11(20)	0.5 mcg/ml	8(53.3)
128 mcg/ml	18(32.7)	1 mcg/ml	-
≥256 mcg/ml	20(36.3)	2 mcg/ml	2(13.3)

the total isolates 55(78.5%) were meropenem resistant and 15(21.5%) were meropenem sensitive. our study results are similar with other studies conducted by Marquez P et al. [15] ,Shanmugam P et al. [16], Seibert et al. [17], Praveen et al. [18].

In the present study most of the isolates were recovered from specimens obtained from ICU patients, 54 (77%), followed by patients admitted in IPD 14 (20%) and least from patients attending OPD 2(3%). In a study conducted by Nayak S et al in Gujarat, the majority of ertapenem resistant i.e. 21 out of 31. (67.74%) *Klebsiella pneumonia* were isolated from ICUs ((MICU, SICU and NICU) followed by wards [19]. Similarly according toJ. Yang et al. 48 non-duplicated KPC-2-producing *K. pneumoniae* strains were isolated from 44 patients, which covered three ICUs and one surgical and medical ward. All of them were positive for Hodge test [20]. Also according to Bhatt et al, most of the resistant isolates were obtained from acute wards (42.9%) and intensive care units (ICUs) (29.5%), followed by other wards (23.2%) and the outpatient department (OPD) (4.4%) [21]. Multidrug resistant gram-negative bacilli are frequently associated with infections in the patients admitted to intensive care units of hospitals. *Klebsiella pneumoniae* has been identified as one of the most frequent causes of outbreaks reported in neonatal intensive care units (NICUs). It is a known cause of sepsis and had been reported in other studies as the commonest blood culture isolates [22].

Minimum Inhibitory Concentration (MIC) of the isolates by Broth microdilution test. For (36.3%) isolates MIC was ≥256 µg/ml followed by 128 µg/ml in (32.7%) isolates followed by 64 in (20%) isolates and 32 in (10.9%). Also mic by vitek 2 for these isolates was ≥16µg/ml. On comparison of susceptibility of meropenem by Vitek 2 and BMD it was found that isolates that were resistant by Vitek-2 were resistant by BMD also.Also according to a study by April M. Bobenchik et al. all 25 CRE isolates evaluated were meropenem and imipenem non-susceptible (intermediate [I] or resistant [R]) on the Vitek 2, regardless of whether the MICs were interpreted by the CLSI M100-S24 or Vitek 2 breakpoints. This improved performance may be attributed to bioMérieux updates to the Vitek 2 software and reformulation of imipenem [23].

Among 15 meropenem sensitive isolates by Vitek-2, 13(86.6%) isolates had MIC in susceptible range by BMD. Only 2 isolate (13.4%) had MIC in nonsusceptible range (intermediate, MIC of 4 µg/ml). A possible reason for the discrepancy in susceptibility resultsamong automated systems might involve the inoculum size. Astudy with the Micro Scan system by Bratu and colleagues demonstrated false susceptibility rates for *Klebsiella pneumoniae* isolates that were proposed to be due to a low inoculum size [24]. Thisproblem has also been reported with the Vitek- 2 system thus leadingto the conclusion that low inoculum size has a major influence on the outcomes of these automated systems, with false susceptibilities being reported [25].

5. CONCLUSION

Overall, the Vitek 2 performance was comparable to that of BMD(no very major and major error) for testing a limited number of *Klebsiella pneumoniae* isolates. Nonetheless, further studies with larger collections of isolates are required to assess the performance of the Vitek- 2 to accurately report MICs in meropenem.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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