Comparision 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.

Original Research Article

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.
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 pneumoniae* (KPC) worldwide is of growing concern [2]. Therefore, monitoring of the development of resistance against carbapenems is necessary [3].

*Klebsiella pneumoniae* is one of the most important gram negative bacterial pathogens 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 infections, 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 antimicrobial agent [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), 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. The inocula prepared were processed by Vitek-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

\[ V1C1 = V2C2 \quad (V1=volume \text{ of starting solution needed, } C1=\text{concentration of starting solution needed, } C2=\text{final concentration of new solution}, \ V2=\text{final volume of new solution}) \]

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. From the 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 study was 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 | 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 (intermediate [I] or resistant [R]) on the Vitek 2, were meropenem and imipenem non-susceptible. 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].

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 results among automated systems might involve the inoculum size. A study 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]. This problem has also been reported with the Vitek 2 system thus leading to 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.

REFERENCES

1. Nicasio AM, Eagye KJ, Nicolau DP, Shore E, Palter M, Pepeetal J. Pharmacodynamic-based clinical pathway for empiric antibiotic choice in patients with ventilator-associated pneumonia. J. Crit. Care. 2010;25:69-77.
2. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect. Dis. 2009;9:228–236.
3. Serapsüzüyıldız, banuka, skatepe, havvaavcıkçük, sükranoztürt. Performance of carbapen and cim tests in oxa-48 carbapenemase-producing Enterobacteriaceae. Acta Microbiologicaet Immunologica Hungarica. 2017;64(1):9–16.
4. Singh M, KakatiB, AgarwalRK, Kotwal A. Detection of Klebsiella pneumoniae carbapenemases (KPCs) among ESBL/MBL producing clinical isolates of Klebsiella pneumoniae. Int. J. Curr. Microbiol. App. Sci. 2015;4(4):726-731.
5. Doern CD, Dunne WM, Jr, Burnham CA. Detection of Klebsiella pneumoniae Carbapenemase (KPC) production in non-
Klebsiella pneumonia, Enterobacteriaceae isolates by use of the Phoenix, Vitek 2 and disk diffusion methods. J Clin Microbiol. 2011;49:1143-1147.

6. Lat A, Clock SA, Wu F, Whittier S, Della-Latta P, Fauntleroy Ketal. Comparison of polymyxin B, tigecycline, ceftazidime and meropenem MICs for KPC-producing Klebsiella pneumonia by broth microdilution, Vitek 2 and Etest. J Clin Microbiol. 2011;49:1795-1798.

7. Bulik CC, Fauntleroy KA, Jenkins SG, Abuali M, LaBombardi VJ, Nicolau DP, et al. Comparison of meropenem MICs and susceptibilities for carbapenemase-producing Klebsiella pneumonia isolates by varying testing methods. J Clin Microbiol. 2010;48:2402-2406.

8. Davies TA, Marie Queenan A, Morrow BJ, Shang W, Amsler K, He W, et al. Longitudinal survey of carbapenem resistance and resistance mechanisms in Enterobacteriaceae and non-fermenters from the USA in 2007-09. J Antimicrob Chemother. 2011;66(10):2298-307.

9. Hentschke M, Gortizka V, Campos CB, Merkel P, Ichmann C, Lellek H, et al. Emergence of carbapenemases in Gram-negative bacteria in Hamburg, Germany. Diagn Microbiol Infect Dis. 2011;71(3):312-5.

10. Mayer G. Antibiotics – protein synthesis, nucleic acid synthesis, and metabolism. In: Microbiol. Immunol. Hunt RC (Ed.). University of South Carolina, SC, USA; 2016.

11. Sievert DM, Ricks P, Edwards JR, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect. Control Hosp. Epidemiol. 2013;34(1):1-14.

12. CLSI Document, M100-S29, Wanye, PA:CLSI. Clinical And Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; Twenty-Ninth Information Supplement; 2019.

13. Ramana KV, Rao R, Sharada V, Kareem MA, Reddy LR, Ratna Mani MS. Modified Hodge test: A useful and the low cost phenotypic method for detection of carbapenemase producers in Enterobacteriaceae members. Journal of Natural Science, Biology and Medicine. 2013;4(2):346-48.

14. Duggal S, Gaind R, Tandon N, Deb M, Chugh T. Comparison of an automated system with conventional identification and antimicrobial susceptibility testing. ISRN Microbiology. 2012;1-4.

15. Marquez P, Terashita D, Dassey D, Mascola L. Population-based incidence of carbapenem-resistant Klebsiella pneumoniae along the continuum of care, Los Angeles County. Infect Control Hosp Epidemiol. 2013;34(2):144-50.

16. Shanmugam P, Meenakshisundaram J, Jayaraman P. Blak PC gene Detection in clinical Isolates of Carbapenem resistant Enterobacteriaceae in a Tertiary Care Hospital. J Clin Diagn Res. 2013;7(12):2736-8.

17. Seibert G, Hörner R, Meneghetti BH, Righi RA, Frasson NA, Forno D, et al. Nosocomial infections by Klebsiella pneumonia carabenemase producing enterobacteria in a teaching hospital Einstein (São Paulo), International Journal of Basic and Applied Medical Sciences. 2014;12:2277-2103.

18. Parveen RM, Harish BN, Parija SC. Emerging carbapenem resistance among nosocomial isolates of Klebsiella pneumoniae in South India. Int J Pharma Biosci. 2010;1(2):1-11.

19. Nayak S, Singh S, Jankhwala S, Pradhan R. Prevalence, characterization and clinical significance of Klebsiella pneumoniae carbapenemase (KPC) producing Klebsiella pneumoniae. Int J Med Res Sci. 2014;3(4):797-803.

20. Yang J, Ye L, Guo L, Zhao Q, Chen R, Luo Y, et al. A nosocomial outbreak of KPC-2-producing Klebsiella pneumoniae in a Chinese Hospital: dissemination of ST11 and emergence of ST37,ST392 and ST395. ClinMicrobiol Infect. 2013;19: E509–E515.

21. Bhatt P, Tandel K, Shete V, Rathi KR. Burden of extensively drug-resistant and pandrug-resistant gram-negative bacteria at a tertiary-care centre. New Microbe and New Infect. 2015;8:166-170.

22. Khan F, Siddiqui N, Sultan A, Rizvi M, Abqari S, Shukla I, et al. Klebsiella pneumoniae outbreak in paediatric ward: Detection and prevention. International Journal of Current Microbiology and Applied Sciences. 2015;1:81-87.
23. April M. Bobenchik, Eszter Deak, Janet A. Hindler, Carmen L. Charlton, Romney M. Humphries, et al. Performance of Vitek 2 for Antimicrobial Susceptibility Testing of Enterobacteriaceae with Vitek 2 (2009 FDA) and 2014 CLSI Breakpoints. J. Clin Microbiol. 2015;53(3):816-823.

24. Bratu S, Mooty M, Nichani S, Landman D, Gullans C, Pettinato B, et al. Emergence of KPC-possessing Klebsiella pneumoniae in Brooklyn, New York: Epidemiology and recommendations for detection. Antimicrob. Agents Chemother. 2005;49:3018–3020.

25. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, et al. Rapid spread of carbapenem resistant Klebsiella pneumonia in New York City. Arch. Intern. Med. 2005;165:1430–1435.

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