

Diagnostic Accuracy of the Carba NP in Clinical Isolates at a Tertiary Care Centre from Pakistan – a cross sectional study

Fizza Farooqui, Seema Irfan

Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Karachi, Pakistan

Abstract

Background

The isolation of carbapenem resistant enterobacteriaceae has increased in frequency with time. Rapid detection methods are important for both treatment and infection control purposes. The Carba NP is one such recently developed rapid chromogenic test. This is one of the first studies from Pakistan that assesses the performance of this test in clinical enterobacteriaceae isolates.

Results

Fifteen isolates each of carbapenem resistant and sensitive isolates were subjected to testing of minimum inhibitory concentration (MIC) of meropenem and the Carba NP test. Sensitivity and specificity of Carba NP were found to be 100% and 93.3% respectively.

Conclusion

The Carba NP is a useful tool for the early detection of carbapenem resistant enterobacteriaceae and activation of infection control response. More studies from the region are needed, especially those that calculate its diagnostic accuracy taking PCR as gold standard so that laboratories can incorporate the test into practice.

Keywords

Carba NP, Minimum inhibitory concentration, carbapenem resistant enterobacteriaceae, Pakistan

Background

The world has noticed an increasing rate of drug resistant pathogens ever since antibiotics have come into use. Broader spectrum antibiotics are used for infections caused by these organisms, and resistance to these antibiotics results in complications in the selection of further antibiotics and prevention of spread of these organisms.¹⁻³ In enterobacteriaceae, carbapenems are used very commonly in hospitals when strains resistant to extended spectrum beta lactams are encountered.⁴

Carbapenem resistant enterobacteriaceae (CRE) are a serious threat and leave antibiotics such as polymyxins as last resort once they are encountered.⁵

Center for disease control and surveillance (CDC), recommends contact precaution and isolation or cohorting of hospitalized patient if CRE are encountered,⁶ while the World Health Organization (WHO) has put them in the critical category on their global priority list of antibiotic resistant bacteria.⁷ Spread of the organism within a health facility is dangerous and therefore, laboratories need to have a proactive approach in alerting the respective departments or clinical areas when CRE are isolated from patients admitted there.⁸

In most developing countries, molecular tests are difficult and expensive to perform in situations when the suspicion of CRE infection arises, and routine culture methods take two to three days to confirm resistance. Therefore, a rapid test that is both accurate and cost effective is required. The Carba NP is a rapid chromogenic test that was developed by Nordmann and Poirel in 2012,⁹ and incorporated in the Clinical Laboratory Standards Institute (CLSI) guidelines in 2015.¹⁰ It detects the hydrolysis of imipenem in a solution of lysed organisms with an indicator (phenol red) that is sensitive to the pH of the medium, and changes its color from red to yellow once the medium turns acidic. Previous studies performed in other parts of the world have found the test to be highly sensitive and specific^{9, 11,12} which suggests that this test needs to be evaluated in our part of the world as well.

To our knowledge, the Carba NP test has not been studied in Pakistan. This study aims to evaluate the sensitivity, specificity and diagnostic accuracy of the Carba NP test taking meropenem minimum inhibitory concentrations (MIC) as gold standard, in a tertiary care laboratory in Pakistan.

Materials and Methods

This study was carried out at the Aga Khan University Hospital in January 2016, non-probability consecutive sampling technique was used. Sample size was calculated using OpenEpi, version 3, open source calculator for unmatched case control study, taking sensitivity of the Carba NP as 99.9%.⁹

The organisms were identified using gram stain and biochemical

Correspondence Author: Seema Irfan,
Department of Pathology and Laboratory Medicine,
Aga Khan University Hospital,
Karachi, Pakistan.
Email: seema.irfan@aku.edu

tests, and disc diffusion was used for initial susceptibility testing, taking zone diameters of ≥ 23 mm against meropenem as sensitive, 20-22 mm as intermediate and ≤ 19 mm as resistant, using CLSI guidelines.¹⁰ An equal number of isolates sensitive and resistant to meropenem were selected.

MIC was calculated using an automated system VITEK 2@ GN81 (broth microdilution) and interpreted according to CLSI guidelines.

The Carba NP was performed according to CLSI guidelines;¹⁰ colonies of the test organism were mixed in a solution containing a cell lysis reagent, impenem, and phenol red, which changes color when the pH turns acidic. After two hours of incubation, a color change from red to yellow was taken as indicative of carbapenemase production.

Statistical analysis: Sensitivity, specificity and diagnostic accuracy after cross tabulation of results and calculation of true and false positive and true and false negative results taking meropenem MIC as the reference method for resistance. SPSS v22.0 was utilized for data entry and analysis.

Results

A sample size of 30 was achieved using the aforementioned method, and 15 isolates each of sensitive and resistant type were selected for the study.

Of the isolated enterobacteriaceae, 26 were isolated from urine and four from blood. Seventeen isolates were identified as *E. coli*, 10 as *Klebsiella* species, and one each as *Enterobacter*, *Citrobacter* and *Serratia* species.

Results of meropenem susceptibility and Carba NP are presented below in table 1; sensitivity and specificity were found to be 100% (95% CI 78.20 - 100%) and 93.3% (68.05% CI 78.20 – 99.83%) respectively. Diagnostic accuracy of this test was found to be 96.6% (CI 82.78-99.92%).

Discussion

Our study confirms the findings of previous studies in which the Carba NP was found to be highly accurate.^{9,11,12} There have been previous studies from Pakistan in which other phenotypic tests such as the Modified Hodge Test (MHT) have been

evaluated,¹³ but to our knowledge, this is the first study from Pakistan evaluating the Carba NP test.

The Carba NP takes two hours and a maximum of three from setting up to interpretation and thus gives very rapid results that can be clearly understood.⁹ This gives a clear advantage over other phenotypic methods such as the EDTA disc synergy test (EDTA DST), MHT, or the modified carbapenem inactivation method (mCIM), which, though simple to perform, take a longer time to be reported.¹⁰

A good microbiology laboratory informs the hospital when there is suspicion of a drug resistant pathogen so that treatment and infection control measures can be implemented.⁸ The importance of a rapid test comes into play when these results are confirmed and the practice is continued, and even more when they are negated resulting in discontinuation of infection control measures and stopping of a broader spectrum antibiotic protecting the patient from added cost and potential side effects.⁶

This study has some limitations. First, that the sample size is very small. Second, that the gold standard taken for calculation of sensitivity and specificity was MIC, which can only confirm carbapenem resistance or susceptibility and not the presence of a carbapenemase.

Therefore, we conclude that the Carba NP is a rapid and accurate test that can be used by laboratories when suspected CRE are encountered. However, larger comparative studies that can confirm results by PCR are needed from the country to prove the utility of this test on a regular basis.

References

- Iovleva A, Doi Y. Carbapenem-resistant Enterobacteriaceae. *Clin Lab Med* 2017 Jun 1;37(2):303-15.
- Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm!. *Trends Mol Med* 2012 May 1;18(5):263-72.
- Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010 Sep 1;10(9):597-602.
- McLaughlin M, Advincola MR, Malczynski M, Qi C, Bolon M, Scheetz MH. Correlations of antibiotic use and carbapenem resistance in Enterobacteriaceae. *Antimicrob Agents Chemother* 2013 Oct 1;57(10):5131-3.
- Livermore DM, Warner M, Mushtaq S, Doumith M, Zhang J, Woodford N. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int J Antimicrob Agents* 2011 May 1;37(5):415-9.
- Facility Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE) November 2015 Update - CRE Toolkit. National Center for Emerging and Zoonotic Infectious Diseases Division of Healthcare Quality Promotion
- WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.
- Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev* 1993 Oct

Table 1: Results of meropenem susceptibility and Carba NP.

	Meropenem resistant	Meropenem susceptible
Carba NP positive	15	1
Carba NP negative	0	14

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- 1;6(4):428-42.
9. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase producing Enterobacteriaceae. *Emerg Infect Dis* 2012 Sep;18(9):1503.
 10. CLSI, Performance Standards for Antimicrobial Susceptibility Testing. 27 ed. Clinical Laboratory and Standards Institute. Vol. M100. 2015, Wayne, PA.
 11. Tijet N, Boyd D, Patel SN, Mulvey MR, Melano RG. Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2013 Jul 1: AAC-00878.
 12. Vasoo S, Cunningham SA, Kohner PC, Simner PJ, Mandrekar JN, Lolans K, Hayden MK, Patel R. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test with the modified Hodge test for detection of carbapenemase producing gram-negative bacilli. *J Clin Microbiol* 2013 Jul 3: JCM-00965.
 13. Sultan, BA, Khan E, Hussain F, Nasir A, Irfan S. Effectiveness of Modified Hodge Test to detect NDM-1 Carbapenemases: an experience from Pakistan. *J Pak Med Assoc* 2013. 63(8): p. 955-960.
 14. Poirel L, Nordmann P. RAPIDEC® CARBA NP test for rapid detection of carbapenemase producers. *J Clin Microbiol* 2015 Jun 17: JCM-00977.
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