EVALUATION OF A RAPID TEST TO DETECT CARBAPENEMASE PRODUCTION AMONG GRAM-NEGATIVE BACILLI

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INTRODUCTION

Carbapenem-resistant Gram-negative bacilli have been emerging globally at increasing rates. Carbapenem sensitivity of Pseudomonas aeruginosa strains isolated from patients with urinary tract infection was only 37% in a study conducted in South India [1]. With limited treatment options and the high morbidity and mortality associated with infections caused by them, it is a huge concern requiring immediate scientific attention. Carbapenem resistance due to non-carbapenemase-mediated mechanisms includes outer membrane permeability defect, efflux mechanisms, loss of porins associated with overproduction of cephalosporinase, and/or extended-spectrum β-lactamases or from strains that are carbapenem susceptible but express a broad-spectrum β-lactamase without carbapenemase activity, extended-spectrum β-lactamase, and plasmid- and chromosome-encoded cephalosporinases [2]. Carbapenemase production is coded on plasmids and transferred from one bacterium to the other very easily. There is a need for an early detection of carbapenemase-producing Gram-negative bacilli to reduce the morbidity and mortality associated with it. The objective of this study is to evaluate the usefulness of the Carba NP test in detecting carbapenemase-producing Gram-negative bacilli.

METHODS

This prospective experimental study was conducted in 2013 at PSG Institute of Medical Sciences and Research, Coimbatore. In this study, 120 consecutive Gram-negative bacilli clinical isolates identified as carbapenem resistant as per the Clinical and Laboratory Standard Institute guidelines were subjected to Carba NP.

RESULTS

About 62.5% of the carbapenem-resistant isolates were found to be carbapenem producers by modified Hodge test. The sensitivity of the Carba NP test was found to be 92% and the specificity was 100%. The most common bacterial isolate was Acinetobacter baumannii, and the most common sample was urine.

Conclusion: The Carba NP test is a simple inexpensive, rapid, reproducible, sensitive test that can be performed directly from the colonies. It improves early detection of patients infected or colonized with carbapenemase in the hospital.

Keywords: Carba NP test, Carbapenemase producer, Modified Hodge test.

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ABSTRACT

Objective: There is a need for an early detection of carbapenemase-producing Gram-negative bacilli to reduce the morbidity and mortality associated with it. The objective of this study is to evaluate the usefulness of the Carba NP test in detecting carbapenemase-producing Gram-negative bacilli.

Methods: This prospective experimental study was conducted in 2013 at PSG Institute of Medical Sciences and Research, Coimbatore. This study, 120 consecutive Gram-negative bacilli clinical isolates identified as carbapenem resistant as per the Clinical and Laboratory Standard Institute guidelines were subjected to Carba NP.

Results: About 62.5% of the carbapenem-resistant isolates were found to be carbapenem producers by modified Hodge test. The sensitivity of the Carba NP test was found to be 92% and the specificity was 100%. The most common bacterial isolate was Acinetobacter baumannii, and the most common sample was urine.

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was the predominant organism 47.5% (57), followed by Klebsiella pneumoniae (29), P. aeruginosa (21), E. coli (8), Enterobacter sp. (3), Proteus sp. (1), and Citrobacter sp. (1) as mentioned in Table 1.

We found that 62.5% (75) of the isolates were positive by MHT. The sensitivity of the Carba NP test was found to be 92% (69), and the specificity was 100% (Table 2). The positive predictive value of Carba NP test was 100%, and the negative predictive value was 88.24%.

**DISCUSSION**

Carbapenems, the last-line drug of therapy, are increasingly reported to be resistant to Gram-negative bacilli causing severe infections. Early detection of these organisms is important to initiate appropriate therapy and for the implementation of infection control practices. In our study, we found 62.5% of the carbapenem-resistant organisms as carbapenemase producers by MHT. Other isolates might be carbapenem resistance due to non-carbapenem-mediated mechanisms. Among many mechanisms of carbapenem resistance, carbapenemase production is the most important one.

The sensitivity and specificity of the Carba NP test were described to be 100% by Dordet Poirel and Nordmann et al. However, in our study, we found the sensitivity to be low (92%). Similar to results in our study, evaluation of Rapidec Carba NP test, a commercially available test for detection of carbapenemases in Enterobacteriaceae derived from the Carba NP-I assay, had sensitivity and specificity of 90.2% and 100%, respectively, when strictly followed the manufacturer’s instructions [5]. In our study, the type of carbapenemase produced was not studied. Carba NP test I developed by Dortet et al. enabled to identify carbapenemase production in Gram-negative bacilli and also to discriminate between the different types of carbapenemases. It is based on the detection of the acidification resulting from imipenem hydrolysis, coupled with tazobactam and ethylenediaminetetraacetic acid (EDTA) as inhibitors [6]. In our study, 3 mg/ml concentration of imipenem and supernatant obtained after centrifuging the lysed bacterial cells was used for the final reaction. Vasoo et al. utilized whole bacterial cells instead of supernatant obtained from bacterial lysis and an increased concentration of imipenem (6 instead of 3 mg/ml) and found that their approach enabled detection of carbapenemase-producing *Pseudomonas* species isolates which were initially negative when tested with 3 mg/ml of imipenem and supernatant obtained after bacterial lysis [7]. Apart from the use of supernatant and an increased concentration of imipenem, sensitivity of the test can be affected by extraction process, expression of gene, hydrolyzing ability of the carbapenemase, stability of carbapenem, inhibitors present, and the pH of the solution. Of 36 isolates of carbapenemase-producing *A. baumannii*, only 33 isolates were detected as carbapenemase producers by Carba NP test in our study. A modified version of Carba NP test and CarbAcineto test, was developed by Dortet et al. to enhance the detection rates of carbapenemase-producing *Acinetobacter* species by replacing the lysis buffer by a 5 M NaCl solution, avoiding any buffer effect, and doubling the bacterial inoculum from one-third to one-half of a calibrated loop (10 µl) to a whole calibrated loop to increase the enzyme quantity [8].

The huge advantage of Carba NP test is that the interpretable positive results were obtained in <2 h, making it possible to implement rapid containment measures to limit the spread of carbapenemase producers. Carba NP test may be useful in particular for intensive care unit and burns patients, among whom multidrug-resistant isolates are widespread. It offers a cost-effective solution for detecting carbapenemase-producing Gram-negative bacilli and preventing their spread, considering that they may harbor those carbapenemase producing genes on plasmids that can spread to other bacterial families (Enterobacteriaceae and the family that includes *Acinetobacter* species). The limitation of the study is that molecular diagnosis of carbapenemase producer and typing of carbapenemases were not included in the study. Combination therapy is recommended for carbapenem-resistant Gram-negative organisms. However, colistin-carbapenem combination therapy has been observed to have low clinical success rate probably due to different interactions between them [9].

**CONCLUSION**

Including this sensitive and rapid test in routine evaluation of the bacterial isolates can initiate prompt treatment and aggressive infection control measures to prevent its spread in the health-care facility.

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**REFERENCES**