A comparative study of Modified Hodge test and Carba NP test for detecting carbapenemase production in gram negative bacteria

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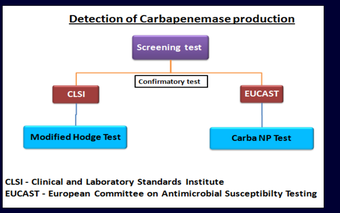
Introduction

- Carbapenemases are β-lactamases that hydrolyze penicillins, in most cases cephalosporins, and to varying degrees carbapenems and monobactams.[1]
- Carbapenemase production in gram negative bacteria (GNB) is being reported globally at increasing rates. Carbapenems are very much effective in treating healthcare associated bacterial infections. For this reason, resistance to carbapenems poses a major challenge to the healthcare system.
- Detection of gram negative bacteria (GNB) that produce carbepenemase has epidemiological significance in controlling further transmission.
- Laboratory strategies for carbapenemase detection in routine AST consist of a screening test and confirmatory test. CLSI recommends Modified Hodge test (MHT) and EUCAST recommends Carba NP test as confirmatory test.
- The MHT is a phenotypic method for detection of carbapenemase production. This test has drawbacks of lack of specificity, a long turnaround time, and poor sensitivity for metallo-β-lactamase detection.
- A rapid chromogenic carbapenemase detection assay, the Carba NP test, based on hydrolysis of the β-lactam ring of imipenem, was described by Patrice Nordmann, Laurent Poirel and Laurent Dortet, first published in 2012.[2] The change of pH due to in vitro hydrolysis of a carbapenem leads to visible colour change from red to yellow/ orange with phenol red indicator.
- The cases of carbapenemase producers missed with Carba NP test has been found by molecular analysis to be mainly of OXA-48-like producers, whereas MHT is less reliable to detect others as well.[3]
- Several modifications were done since first publication : [4,5,6] whole bacterial cells rather than supernatant after lysis; eppendorf tubes rather than microtitre tray; increased concentration of imipenem.



Objective

This study was designed to evaluate the efficacy of the MHT and Carba NP test for the detection of carbapenemase production on well-characterized gram negative bacilli.

Materials & Methods

- Clinical samples (urine, pus, wound swab etc.), collected from patients from June'2014 to May'2015, were inoculated in suitable selective and non selective culture media.
- After overnight incubation at 37°C, culture media were examined for bacterial growth and gram stain was done from bacterial colonies. Antibiogram of each GNB was carried out following the CLSI guidelines, on Mueller-Hinton agar (MHA) using Meropenem 10 µg (by Kirby Bauer's disk diffusion technique). Motility test and biochemical tests were done for identification of the GNB. The media were incubated at 37°C. On the next day, GNB strains which showed reduced susceptibility to Meropenem were selected for the study. The strains were subjected to MHT (according to CLSI guideline) and Carba NP test (as per the procedure described herewith).
- A total of 90 well characterized GNB strains were used for this study, which included 68 *Enterobacteriaceae* and 22 non-fermenting GNB.
- The number of screened strains which were MHT positive and those which showed presence of carbapenemase activity by Carba NP test were recorded.
- For Modified Hodge test - MHA plates were examined following overnight incubation at 37°C for enhanced growth around the test or control organism streak at the intersection of the streak and the zone of inhibition (Figure 1). Enhanced growth was considered as positive for carbapenemase production. No enhanced growth was considered negative for carbapenemase production.
- Carba NP test was read after two hours to find out the change of colour of the solution. Carbapenemase producing strains caused change of colour from red to yellow or orange. Strains that did not produce carbapenemase remained red (Figure 3).
- The number of screened strains which were MHT positive and those which showed presence of carbapenemase activity by Carba NP test were recorded.

Figure 1 : Modified Hodge test

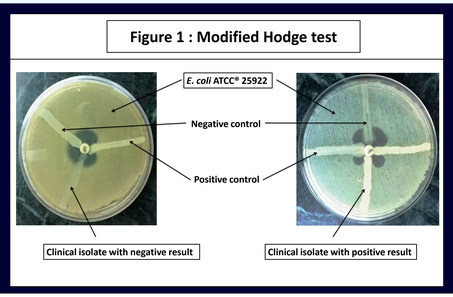


Figure 2 : Carba NP test

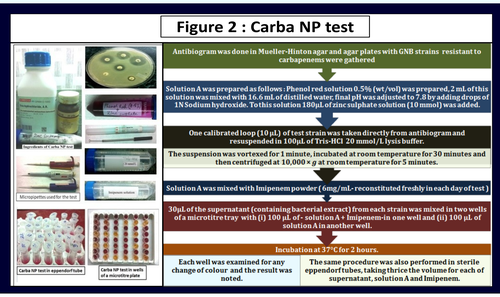
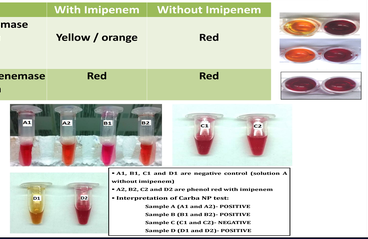


Figure 3 : Results of Carba NP test

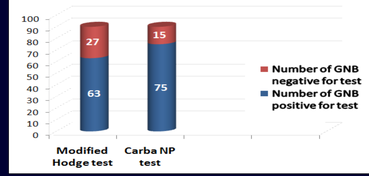
	With Imipenem	Without Imipenem
Carbapenemase production	Yellow / orange	Red
No carbapenemase production	Red	Red



Results and Discussion

- Out of the ninety GNB strains screened resistant for carbapenems during the study period, 63 (70%) were positive for MHT and 75 (83%) showed presence of carbapenemase activity by Carba NP test (Table 1).
- In our study, the advantage of the Carba NP test were : visible colour change leading to simple interpretation ; easy to perform in clinical laboratory; no costly reagent or special instrument is required; no special skill or training is required. Moreover, it is a rapid test where the results can be read in short time.
- But there are some limitations of Carba NP test, which are : variations in concentration of available reagents and technical variation between laboratories may lead to variations in result. Molecular analysis is needed for validation of test results.

Table 1 : Distribution of GNB strains according to Modified Hodge test and Carba NP test results



Conclusion

- The major advantage of Carba NP test was short turnaround time than MHT.
- Since the number of detected carbapenemase producers were significantly higher by Carba NP test, it appears that the CLSI recommended MHT fails to confirm many carbapenemase producers.
- But this observation of the study can only be validated by molecular analysis of the strains.

References

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