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Modified Hodge Test and Remodified Hodge Test for Carbapenemase Detection: Better Indicator

KEYWORDS: Carbapenemase, Modified Hodge test, Remodified Hodge test

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ABSTRACT *Purpose: Study to detect carbapenemase by two different phenotypic methods*
Methods: A total of 200 Gram negative over period of 12 months were included in the study. These isolates were then subjected to modified hodge test (MHT) and remodified hodge test.
Results: Of the 200 isolates, 56 isolates were positive for either the MHT or the remodified Hodge test. Eleven isolates which were negative by MHT were positive by remodified Hodge test and 45 isolates had an enhanced indentation in the remodified Hodge test compared with MHT
Conclusion: Carbapenem resistance mediated by MBL should be further chacterised only by molecular methods due to cost effective this is not done in this study.

Introduction

A number of gram negative bacilli of clinical importance have acquired resistance to standard antibiotics. Enterobacter spp., Pseudomonas spp., Escherichia coli, Klebsiella spp., and Bacteroids spp. have all acquired resistance to some of the agents that have been historically used in their treatment. Fortunately the means of overcoming resistance are available for nearly all gram negative pathogenic bacilli. In those producing a β lactamase, using a β lactam antibiotic that is relatively β lactamase resistant or combining a β lactam with β lactamase inhibitor can often results microbial sensitivity. Through learning more about the mechanism and epidemiology of resistance towards antimicrobial drugs, it has become clear that bacteria have remarkable array of tools at their disposable to overcome antibiotics. The widespread use of antimicrobial drugs for immunocompromised patient and in the intensive care units of modern hospitals clearly results in the selection of the multidrug resistant organisms that cause serious infection. The prevalence of antimicrobial resistant human pathogen is rapidly increasing, but the recovery and development of new antimicrobial drugs are active against multidrug resistant organisms have slowed dramatically. The extent to which bacteria develop resistance to antimicrobial drugs and the speed with which they do so varies with different types of drugs, so far resistance has developed to all antimicrobials drugs. Moreover, there are increasing frequent reports of clinical problems caused by bacteria resistant to multiple antimicrobial drugs [1].

Materials and Methods

The study was done in Department of Microbiology from tertiary care hospital South India. A total of 200 Gram negative rods from different clinical samples over period of 12 months from October 2011 to October 2012 were included in the study. These isolates were then subjected to modified hodge test and remodified hodge test. Quality control of carbapenem disc was performed according to CLSI guidelines. Quality control of the following organism MHT positive Klebsiella pneuomoniae ATCC 1705 and MHT negative Klebsiella pneuomoniae were run with the test.

Methods

Modified Hodge test (MHT) [2] [3]

The ATCC E.coli 25922 at the turbidity equivalent to that of 0.5 Mcfarland was inoculated onto Muller Hinton Agar (MHA) (Himedia Laboratories Pvt.Ltd.,India). After brief drying for five minutes, an imipenem (10 µg) disc was placed at the centre of the plate. The test strain was heavily streaked from the edge of imipenem disc to the periphery of the plate. Four isolates were inoculated in one plate at 90˚ to each other and the plates were incubated at 35°C for 18 – 24 hours. Presence of clover leaf type of zone of inhibition near the test organism was interpreted as positive for carbapenemase production.

Remodified Hodge test [4]

Addition of zinc has been known to increase the activity of metallo beta lactamases. A total of 10µl of 50 mM (140µg per disc) of zinc sulphate (Rankem Chemicals, India) solution was added to imipenem disc.MBL production was interpreted as inward indentation of the growth of the indicator strain along the streak line of the test strain. This was read as negative, equivocal, positive and strongly positive.

RESULTS

A total of 200 isolates obtained from various clinical specimens like urine, pus, wound swab, stool, sputum, blood over a period of one year were included in this study. These isolates includes E.coli (61%), Klebsiella spp (18%), Pseudomonas spp(09%), Acinetobacter(04%) , Proteus (04%), Citrobacter(1.5%), Enterobacter(2.5%).Of all the 200 isolates, 56 isolates were positive for either the MHT or the remodified Hodge test. Eleven isolates which were negative by MHT were positive by remodified Hodge test and 45 isolates had an enhanced indentation in the remodified Hodge test compared with MHT. That is there was an enhancement of indentation demonstrating zinc dependence in total of 56 isolates. In the remaining 142 isolates there was no further change or enhanced indentation; remaining two isolates probably produce bacteriocin that inhibited the growth of the indicator strain. Use of other test like combined disc test and double disc synergy test along with MHT will detect strains producing bacteriocin like substances that rendered the MHT non-interpretable. Besides, some strains of Proteus pose difficulty in interpretation of MHT due to swarming.

Table 1: Comparison of phenotypic test for detection of MBL

Phenotypic test	Number of MBL positive isolates	Percentage (%)
Modified Hodge Test	45	22.5
Remodified Hodge Test	58	29

Table 2: Bacteria showing positive results in phenotypic test

S.NO	Name of organism	Modified Hodge Test	Remodified Hodge Test
1	E.coli	17	22
2	Klebsiella spp	12	18
3	Pseudomonas	08	10
4	Acinetobacter	05	05
5	Citrobacter	02	02
6	Enterobacter	01	01

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DISCUSSION

Carbapenem are class of β lactam antibiotic that seen to be the last line drug to combact against gram negative pathogens. Treatment with carbapenem was challenged by various mechanism of resistance linked with mobile genetic elements were alarming for a serious problem in the future. Carbapenem resistance mediated by MBL should be further chacterised only by molecular methods due to cost effective this is not done in this study.

The emergence of Gram negative bacterial species with acquired resistance to various broad spectrum β lactam is becoming a worldwide clinical problem. Use of simple screening test will be crucial step in monitoring these emerging resistant strains[5]. Many studies have suggested Colistin and Tigecycline as the only treatment option. However both these drugs are not treatment option for Proteus spp as they are inherently resistant to them. Two studies in 2006 by Sidjabat et al [5] and Neonakis et al [6] were Tigecycline resistence was observed in klebsiella spp. Various studies have published guidelines for detection and surveillance of carbapenemase producing Enterobacteriaceae[7-9].

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