

Original Research Article

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Comparison of Phenotypic Methods of Detection of Carbapenamases in a Tertiary Care Hospital, South India

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ABSTRACT

Keywords

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Enterobacteriaceae are the most frequently isolated organisms from clinical specimens. Among them *Escherichia coli* and *Klebsiella pneumoniae* cause a wide variety of clinical infections. With the emergence of ESBLs, carbapenems became the drug of choice. During the past decade, there has been an emergence of carbapenems-resistant Enterobacteriaceae that produce carbapenamases. So detection of carbapenamase producing Enterobacteriaceae has become mandatory for effective treatment. Hundred isolates of Meropenam resistant *Escherichia coli* and *Klebsiella pneumoniae* were selected. Modified Hodge test, double disc synergy test, combined disc or Disc enhancement test and Carba Np Test were done for these resistant isolates among them. The sensitivity and specificity of Carba Np test was 100 % and better than Modified Hodge test, combined disc test and double disc synergy test. Thus carba Np test is more effective and easily adoptable method used to identify the carbapenamase producing organisms.

Introduction

Gram negative bacteria belonging to the family of Enterobacteriaceae are the most frequently isolated organisms from clinical specimens. They are widely dispersed in soil, water, plants, they cause a wide variety of clinical infections. Treatment of these infections depends on the site and severity. Resistance produced by these organisms pose a great difficulty in treatment and hence detection of these resistance producing organisms becomes mandatory.

As the name indicates enterobacteriaceae is also present in intestinal tract of human and

animals. The members of this family of Enterobacteriaceae exhibit general morphological and biochemical similarities.

Within the family they exhibit wide biochemical and antigenic heterogeneity. Enterobacteriaceae are inhabitants of wide variety of niches, including the human, animal gastrointestinal tract and various other environmental sites.

The family Enterobacteriaceae includes a large number and diversity of genera. *Escherichia coli* causes urinary tract infections, cystitis, bacteremia, neonatal meningitis, diarrhea and dysentery. *Klebsiella*

causes a wide variety of nosocomial infections of respiratory tract, urinary tract, and other normally sterile sites. They most frequently infect hospitalized and seriously debilitated patients.¹

Treatment of *Escherichia coli* infections depend on the site and severity of the infection. *Escherichia coli* are treated with Ampicillin, third generation cephalosporins like ceftriazone. Doxycycline, TMP/SMZ, fluoroquinolones.

Klebsiella infections are intrinsically resistant to Ampicillin, Ticarcillin and nitrofurantoin is poorly active against them. In Severely ill patients with *Klebsiella* infections, the treatment options include third generation cephalosporins, quinolones and amino glycosides².

Escherichia coli and *Klebsiella* initially responded to betalactam antibiotic but there was a dramatic increase in resistance to betalactam antibiotics due to beta lactamases produced by them.

With the emergence of ESBLs, carbapenems became the drug of choice. During the past decade, there has been an emergence of carbapenems-resistant *Enterobacteriaceae* that produced carbapenamases, enzymes that efficiently hydrolyze carbapenems, as well as most β -lactam drugs³.

Materials and Methods

After Ethical clearance from the institution, the study was conducted between April 2014 - August 2015. Out of 2042 *Escherichia coli* and 1192 samples of *Klebsiella pneumoniae* isolated from clinical samples, 560 samples of *Klebsiella pneumoniae* (47%) and 148 isolates of *Escherichia coli* (7%) were resistant to carbapenems (Meropenam) by disc diffusion method. Hundred isolates of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* were selected randomly for comparison of phenotypic methods like.

1. Modified Hodge test
2. Double disc synergy test
3. Combined disc or Disc enhancement test
4. Carba Np Test

Results and Discussion

It was observed that 42% were positive by Double disc diffusion test, 75% were positive by Combination disc test, 90% were positive by Modified Hodge test and 98% were positive by Carba Np test. The sensitivity and specificity of Carba Np test is 100 % and is better than Modified Hodge test (sensitivity 91% specificity 100%), Combined disc test (sensitivity 92% and specificity 50%) and double disc synergy test (sensitivity 42% and specificity 50%) (Fig. 1–3 and Table 1).

Table.1

S.No	Name of the test	Positives	Negatives	Sensitivity	Specificity
1	Double disc synergy test	42	58	42	50
2	Combination disc test	75	25	92	50
3	Modified Hodge test	90	10	91	100
4	Carba NP test	98	2	100	100

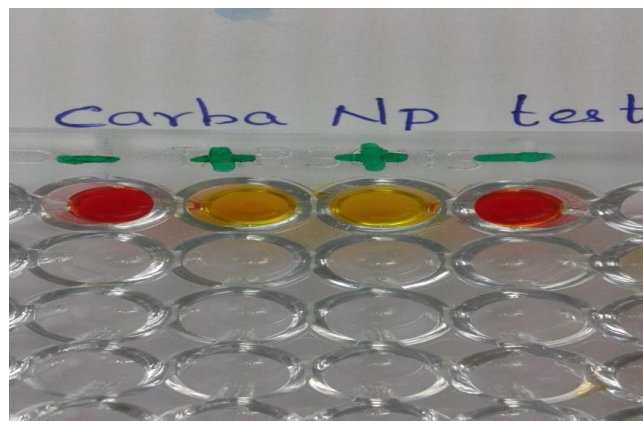
Fig.1 Double disc synergy test and combination disc test



Fig.2 Modified Hodge test



Fig.3 Carba NP test



Enterobacteriaceae are inhabitants of wide variety of niches, including the human, animal gastrointestinal tract and various other environmental sites. The family Enterobacteriaceae includes a large number

and diversity of genera. *Escherichia coli* and *Klebsiella pneumoniae* cause sepsis, endotoxic shock, urinary tract infections, meningitis in neonates and pneumonia in immune compromised. They are normally

treated based on the antibiotic sensitivity, site of infection and co morbidity condition. *Escherichia coli* and *Klebsiella* infections initially responded to betalactam antibiotics, but there was a dramatic increase in resistance to beta lactam antibiotics due to betalactamases produced by them. This resistance was superseded by use of second and third generation cephalosporins and combination of betalactam antibiotics with betalactamases inhibitors. Further with the emergence of ESBLs and Amp C resistance, carbapenems became the drug of choice. There has been a rapid increase in carbapenems resistance in Enterobacteriaceae in the recent years. The carbapenems resistance may be due to carbapenamases production, Efflux pump and Amp C enzyme production with membrane impermeability.

The various methods of carbapenem resistance detection are double-disc synergy, Combined disc, Modified Hodge and Carba Np test.

Double disc synergy test was done with Ertapenem, ceftazidime and disc with EDTA in between. Any distortion of zone towards EDTA disc was considered positive. Combination disc test was done with Ertapenem and Ertapenem with EDTA. An increase in zone diameter with combination disc of more than or equal to 5mm was considered as positive. Clover leaf pattern on the Muller Hinton plate with in a lawn ATCC *Escherichia coli* and test strain streaked perpendicular to it indicates positivity..

Carba Np test is found to be the most specific and sensitive method, one calibrated loop of test organism was taken from a MHA plate. The colony was suspended in protein extraction lysis buffer, vortexed for 1minute, incubated at room temperature for 30 minutes and centrifuged at 10,000 rpm for 5minutes. 30 ul of supernatant of centrifuged bacterial

isolate was added to 100ul of a 1ml solution made of 3mg Imipenem monohydrate, pH7.8, phenol red solution and 0.1mmol/l $Znso_4$ and Incubated at 37⁰c for 2 hrs. The colour of wells changed from Red to yellow in all the test strains producing carbapenamases. The colour remained red for the negative strains.

In our study also we found that Carba Np test is most specific (100%) and sensitive (100%) in detecting carbapenamases and it was better than Modified Hodge test (sensitivity 91% specificity 100%), Combination disc test (sensitivity 92% and specificity 50%) and Double disc synergy test (sensitivity 42% and specificity 50%).

In another study on comparison of various phenotypic tests (DDST, CDT, MHT). Modified Hodge test was comparatively more specific and sensitive than Double disc synergy test and combination disc test⁴. Amjad et al observed carbapenamases in 69% of isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Citrobacter diversus* and *Enterobacter agglomerans*) by Modified Hodge test⁵.

In a study on comparison of carbapenemase detection using a novel chromogenic rapid test-the Carba NP test and the modified Hodge test (MHT). Sensitivities were found to be comparable (CNP, 100%, versus MHT, 98%; P 0.08), but Carba Np was more specific (100% versus 80%; P < 0.0001) and faster⁶.

In a similar study Carba NP test detected all carbapenemase producers regardless of the type of the carbapenemase and of their mucoid phenotype with 100% sensitivity and 100% specificity⁷.

Most of Carbapenem resistant strains were from urine samples (47%). A similar study

showed 57.89% carbapenamases producing *Klebsiella pneumoniae* in urine samples⁴. Khanduri *et al.*, detected in his study that among 50 isolates of carbapenem resistant Enterobacteriaceae 44% were *Klebsiella pneumoniae* and 20% were *Escherichia coli*⁸

The various methods of carbapenem resistance detection are double-disc synergy, Combined disc, Modified Hodge and Carba Np test.

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Carba Np test is found to be the most specific and sensitive method to detect carbapenamases production. In our study also we found that Carba Np test is most specific (100%) and sensitive (100%) in detecting carbapenamases and it was better than Modified Hodge test (sensitivity 91% specificity 100%), Combination disc test (sensitivity 92% and specificity 50%) and Double disc synergy test (sensitivity 42% and specificity 50%).

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In conclusion the indiscriminate use of antibiotics led to the development of drug resistance to almost all antibiotics discovered. The development of carbapenem resistance especially in the members of Enterobacteriaceae, which are the commonest etiological agents in various infections. Being a plasmid coded drug resistance it is spreading with enormous speed not only within the country but also in various nations across the globe. Appropriate detection of such emerging mechanism of resistance goes in along way in preventing unnecessary mortality and morbidity among patients. Effective faster and easier methods like carba Np test helps us in earlier detection of these infections.

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