



Original Research Article

Detection of carbapenemase production by rapid carba NP test among Enterobacteriaceae isolates in tertiary care hospital



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ABSTRACT

Introduction: The spread of carbapenemase producers is the most important clinical issue in antibiotic resistance in gram negative bacteria particularly Enterobacteriaceae. There is an utmost importance of rapid detection. Several phenotypic and genotypic tests are present for detection of carbapenemases but are time consuming, require expertise and well established laboratory. Our study aims at detection of carbapenemase production by rapid Carba NP test

Materials and Methods: A prospective study of two months duration was done among 150 Enterobacteriaceae species (Escherichia coli 88, Klebsiella pneumonia 49 and others 13) isolated from various clinical samples in a tertiary care Hospital. Strains were first identified by standard phenotypic methods. Resistance to carbapenems was detected using Ertapenem (10mcg) disk by Kirby Bauer disk diffusion method and Carba NP test as per the CLSI standards. Carba NP test is based on the detection of Imipenem hydrolysis by carbapenemase producing bacteria. Hydrolysis acidifies the medium which results in colour change of the pH indicator.

Results: Among 150 isolates, Carba NP positive 34(22.6%) and negative 116(77.3%). Ertapenem disk diffusion detected 122(81.3%) as susceptible, 8(5.3%) as intermediate and 20(13.3 %) as resistant. Carba NP has a sensitivity (61.76%), specificity (93.97%), PPV (75%), NPV (89.34%), accuracy (86.67%) which are statistically significant with 'p' value <0.05.

Conclusion: CNP detects larger number of carbapenemases within shorter time (<2h) compared to disk diffusion (~16-18h) which is rapid, highly specific, accurate and gives result in single day with minimal reagents.

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1. Introduction

Multidrug resistance is the emerging problem at an alarming rate causing both Nosocomial and community acquired infections.¹ Gram negative bacteria specifically Enterobacteriaceae is the most common cause of community as well as Hospital acquired infections including urinary tract infections, peritonitis, septicemia, pulmonary infections, soft tissue infections and device associated infections. As Carbapenems (imipenem, meropenem, ertapenem and doripenem) are last line of therapy for Extended spectrum β lactamases producing organisms and most frequently required to treat Nosocomial infections, infections due to carbapenem resistant organisms have become a great

concern as it leaves health care system with limited therapeutic options.

Carbapenemases are carbapenem hydrolyzing beta-lactamases that confer resistance to a broad spectrum of beta-lactam substrates including carbapenems. Resistance to carbapenems is mostly mediated by production of carbapenemases, decreased outer membrane permeability and efflux pump mechanisms.² Non carbapenemase related mechanism of carbapenem resistance is not transferable²⁻⁴ whereas Carbapenemase related are transferrable through plasmid and are potentially responsible for outbreaks and are largely associated with multi or pan drug resistance in gram negative bacteria, particularly Enterobacteriaceae.²⁻⁴ Early and rapid detection of carbapenemase producing gram negative bacteria is of utmost importance for reducing

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primary and secondary infections and also helps in the containment of spread of infection.

Various carbapenemases have been reported in Enterobacteriaceae such as: Klebsiella pneumonia carbapenemase (KPC; Ambler class A); Verona integron – encoded metallo β -lactamase (VIM), imipenemase (IMP), New Delhi Metallo β -lactamase (NDM) (all belong to Ambler class B); and oxacillinase-48 (OXA – 48; Ambler class D),^{2,5–7}

Several phenotypic methods like disc diffusion, MIC determination are most widely used in routine diagnostic practices to determine carbapenem susceptibility or resistance pattern. Ertapenem is preferred over Imipenem or Meropenem for invitro susceptibility testing due to its superior sensitivity and has reported to detect most carbapenemase producers.^{8,9} Other tests for screening and detection of carbapenemases such as modified Hodge test which is not highly sensitive and specific^{2,8,10} and molecular detection of carbapenemase genes which are time consuming,^{2,8,11,12} highly expensive, require expertise and well established laboratory.

Nordmann et al.¹³ have developed a biochemical based test Carba NP, which is rapid can detect production of carbapenemase within 2hrs, easy to perform with good sensitivity and high specificity, inexpensive and reproducible.

Though phenotypic methods are routinely done but as they are time consuming. The present study compares the rapid Carba NP test with Ertapenem disk diffusion (DD) method for detection of carbapenemase producers among Enterobacteriaceae.

Clinical Standards Institute (CLSI) recommends Carba NP (CNP) test as confirmatory test for carbapenemase production among Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter species.¹⁴

2. Materials and Methods

A prospective study was conducted for a period of two months among 150 Enterobacteriaceae species (Escherichia coli 88, Klebsiella pneumonia 49 and others 13) isolated from various clinical samples (blood, urine and sputum) in tertiary care Hospital. The study was conducted after obtaining Ethical committee clearance.

Identification of these strains was done using standard phenotypic methods such as Gram's stain, growth characteristics and biochemical reactions. Resistance to carbapenems was detected using Ertapenem (10 μ g) disk by Kirby Bauer disk diffusion (DD) method and Carba NP test developed by Nordmann et al and interpreted as per the CLSI standards.¹³

The chemicals, antibiotic disk and media required for performing the test were procured from Himedia Laboratories, Mumbai. Inject ion Imipenem + Cilastatin for Carba NP test was procured from Ranbaxy laboratories,

Mumbai.

2.1. Ertapenem disk diffusion method

A lawn culture of Enterobacteriaceae isolates was done on Muller Hinton agar. 10 μ g of Ertapenem disk is suspended on the agar surface and incubated at 37°C overnight and interpreted as per Clinical Standards Institute (CLSI) standards.

Susceptible	≥ 22
Intermediate	19-21
Resistant	< 18

Chart 1:

2.2. Carba NP test

2.3. Principle

Carba NP based on the detection of Imipenem hydrolysis by carbapenemase producing bacteria. Hydrolysis acidifies the medium which results in colour change of the pH indicator.

2.4. Reagents

Clinical laboratory reagent water, commercially available bacterial protein extract reagent in Tris HCL buffer, pH 7.4, zinc sulfate heptahydrate, phenol red powder, 1 N NaOH solution, 10% HCL solution, microcentrifuge tubes 1.5ml, 1 μ l inoculation loops.

2.5. Preparation of carba NP solution A

2ml of 0.5ml phenol red solution is added to 16.6ml clinical laboratory reagent water, vortexed and adjusted to a final pH 7.8 by adding NaOH. Phenol red is the pH indicator. Carbapenemase producing strain breaks down imipenem into acidic products which turns the color of the phenol red indicator to yellow. A volume of 180 μ l ZnSO₄ was added to obtain a final concentration of 0.1mM ZnSO₄. ZnSO₄ is added to enhance the activity of metallo-beta-lactamase (MBL) carbapenemases which increases the sensitivity of Carba NP test to detect MBL carbapenemases.

2.6. Preparation of Carba NP solution B

Solution A + 12mg/ml imipenem cilastatin injectable form (equivalent to 6mg of imipenem reference standard powder).

2.7. Procedure

^{13,14} Bacteria grown on Muller Hinton Agar (MHA) is taken with 1 μ l loop and suspended in 1.5ml eppendorf tube

containing 100 μ l of 20mM Tris HCl lysis buffer and mixed using a vortex device for 5s. This lysate is then mixed with 100 μ l of aqueous indicator solution containing 0.05% phenol red with 0.1mmol/liter ZnSO₄, previously adjusted to pH 7.8 and 6mg/ml Imipenem powder or 12mg/ml Imipenem + Cilastatin injectable form (equivalent to 6mg of Imipenem standard powder) is taken as reaction tube or tube “A” and control tube or tube “B” as indicator solution without antibiotic. Tubes are vigorously mixed for 5 to 10s initially both tubes are red or red – orange in colour, incubated at 37°C and monitored for 2h and observed for colour change.

2.8. Interpretation

After 2h, If:

CNP positive – tested isolated is carbapenemase producer.

CNP negative – tested isolated is non carbapenemase producer.

Repeated testing indicated for invalid result.^{13,14}

2.9. Quality control

With every panel of test isolates, quality control strains were tested. *Klebsiella pneumoniae* ATCC BAA-1705 as positive control and *Escherichia coli* ATCC 25922 as negative control.

2.10. Statistical analysis

The collected data were analyzed using statistical package for social sciences (SPSS) software (version 21.0) and Epi – info softwares.

3. Results

Among 150 Enterobacteriaceae isolates, Carba NP positive were 34(22.6%) and negative were 116(77.3%). Ertapenem disk diffusion detected, 122(81.3%) as susceptible, 8(5.3%) as intermediate and 20(13.3%) as resistant.

Out of 116 Carba NP negative strains, 7 are resistant to Ertapenem. Out of 34 Carba NP positive, 13 are sensitive to Ertapenem by disk diffusion method.

Carba NP has a sensitivity (61.76%), specificity (93.97%), PPV (75%), NPV (89.34%), accuracy (86.67 %) which are statistically significant with ‘p’ value <0.05

Table 1: Total number of Enterobacteriaceae isolates. n = 150

	Number	Percentage
<i>Escherichia coli</i>	88	58.6%
<i>Klebsiella pneumoniae</i>	49	32.6%
Others(<i>Citrobacter</i> spp -7, <i>Proteus</i> spp -6)	13	8.6%
Total	150	

Table 2: Susceptibility pattern of Ertapenem disk of all isolates

	Number	Percentage
Sensitive	122	81.3%
Intermediate	8	5.3%
Resistant	20	13.3%
Total	150	

(The Z – score is -8.0829. The p value is 0. The result is significant at p<0.05)

Table 3: Carba NP test results among all isolates

Positive	34	22.6%
Negative	116	77.3%
Total	150	

(The Z – score is -9.4685. The p value is 0. The result is significant at p<0.05)

Table 4: Comparison of Ertapenem disk diffusion with Carba NP (CNP) test.

Ertapenem Susceptibility	CNP Positive	CNP Negative	Total
Resistance	21	7	28
Sensitive	13	109	122
Total	34	116	150

For Ertapenem disk diffusion both resistant and intermediate is has been considered under resistant.

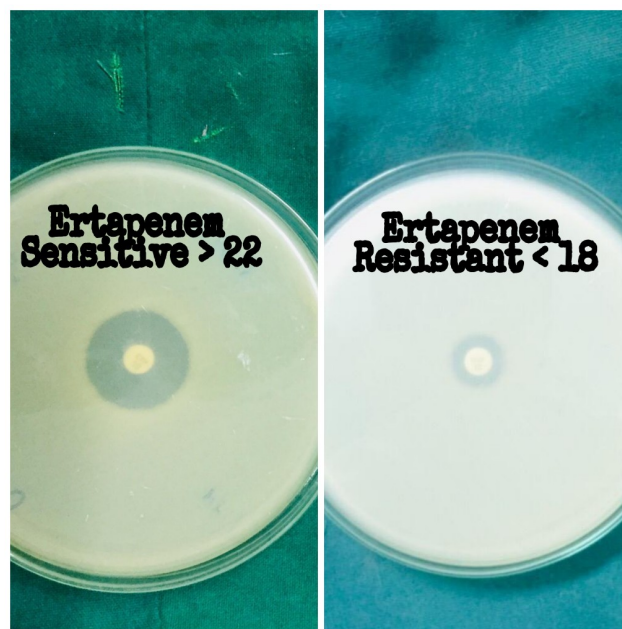


Fig. 1: Photograph showing Ertapenem (10 μ g) disk sensitivity and resistance by Kirby Bauer disk diffusion method.

Results for Patient and QC Tubes		
Tube “a”: Solution A (serves as internal control)	Tube “b”: Solution B	Interpretation
Red or red-orange	Red or red-orange	Negative, no carbapenemase detected
Red or red-orange	Light orange, dark yellow, or yellow	Positive, carbapenemase producer
Red or red-orange	Orange	Invalid
Orange, light orange, dark yellow, or yellow	Any color	Invalid

Chart 2:

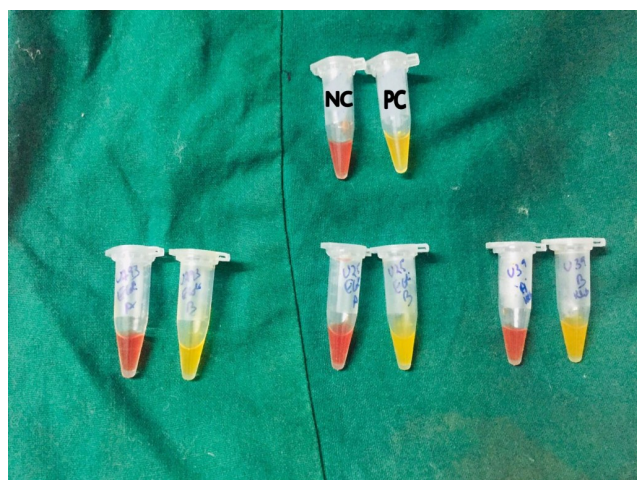


Fig. 2: Picture showing results of Carba NP test with positive and negative controls.

4. Discussion

In the present study total Enterobacteriaceae isolates are 150 among them *Escherichia coli*- 88 (58.6%), *Klebsiella pneumoniae* – 49(32.6%) and others -13(8.6%). All the isolates were tested for rapid Carba NP test and Ertapenem susceptibility by Kirby Bauer disk diffusion method. Similar study was done by Shinde et al who has tested for 400 Enterobacteriaceae among them *Escherichia coli* 244(61%), *Klebsiella pneumoniae* 135(33.75%), others 21(5.25%).

All the 150 isolates of Enterobacteriaceae were first tested for Ertapenem susceptibility by Kirby Bauer disk diffusion method and interpreted as per Clinical Laboratory Standards Institute (CLSI). Out of 150 isolates Ertapenem susceptible were found to be 122(81.3%), intermediate were 8(5.3%) and resistant were 20(13.3 %) which is compared to Shinde et al¹⁵ who had studied for 400 isolates out of which Ertapenem susceptible are 302, intermediate are 16 and resistant are 82.

Ertapenem is preferred over Imipenem or Meropenem for invitro susceptibility testing due to its superior sensitivity (97% vs 42% and 71%)¹⁶ and has reported to detect most

carbapenemase producers as reported by Nordmann et al and Gniadkowski et al in their study.^{8,9}

All the 150 isolates of different Enterobacteriaceae species were tested for carbapenemase production by rapid Carba NP test developed by Nordmann et al. Out of 150 isolated 34(22.6%) were positive, that are carbapenemase producers and 116(77.3%) were negative, non-carbapenemase producers which were compared to Shinde et al¹⁵ who tested Carba NP for 400 isolates, out of which 106 were positive, carbapenemase producers and 294 were negative, non carbapenemase producers.

Carba NP test developed by Nordmann et al⁹ demonstrated that Carba NP has high sensitivity and high specificity when compared with the molecular studies where resistance genes can be detected. Tijet et al¹⁷ in 2013 have also reported excellent specificity but lower sensitivity.

In the present study sensitivity and specificity of Carba NP test is 61.76% and 93.97% respectively which correlates with Tijet et al¹⁷ in 2013 and Vasoo et al¹⁸ who reported an excellent specificity but a lower sensitivity.

Based on the high specificity and negative predictive value of Carba NP test, In the present study 13strains which are Carba NP positive due to carbapenemase production were susceptible by disk diffusion which Ertapenem failed to detect which can lead to adverse consequences in clinical management of the patient which correlates with Shinde et al¹⁵ were 36 strains which are Carba NP positive were falsely susceptible by Ertapenem disk diffusion method.

In another condition 7 strains which were resistant by Ertapenem disk diffusion method was found negative by Carba NP test due to carbapenem resistance other than carbapenemase which correlates with Shinde et al¹⁵ were 28 strains which were resistant by Ertapenem disk diffusion test was tested negative by Carba NP test which may be due to true negativity as a result of carbapenem resistance other than carbapenemases.

The above results indicated that Carba NP test has multiple benefits. It is rapid, cheaper, highly specific and most widely used to identify carbapenemase producers than other phenotypic tests.

In a study done by Galani et al and Habrak et al^{16,19} who has done molecular techniques like real time PCR for detection of carbapenemase gene which however cannot detect the resistance mechanism of carbapenemases and also expensive, requires expertise and well established laboratory making it unsuitable for routine purposes in the laboratories. Whereas Carba NP is easy to perform, rapid (within 2hrs), cheaper, does not require man power, requires minimal reagents and can be performed on routine basis in laboratories for detection of carbapenemase resistance.

5. Conclusion

Carba NP detects larger number of carbapenemases within shorter time (<2h) compared to Ertapenem disk diffusion method (16-18h) which is rapid, gives result in single day with minimal reagents, cheaper, less man power and not labor intensive.³ Carba NP also detects carbapenemase producers where Ertapenem fails to detect which is of clinical significance. The rise in prevalence of Carbapenemase producing strains associated with significant mortality recommends the incorporation of this simple and rapid cost effective test in routine diagnostic laboratories thereby benefitting patient care and antimicrobial stewardship.

6. Source of Funding

None.

7. Conflict of Interest

None.

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