Journal of Advances in Medicine and Medical Research

24(9): 1-11, 2017; Article no.JAMMR.36876
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

CARBA NP a Rapid and Reliable Test for Detection of Carbapenemases in Gram Negative Bacteria

Saba Choudhary[1], Bashir Ahmad Fomda[1*], Munazah Manzoor[1], Gulnaz Bashir[1], Asiya Khan[1] and Mir Yawar[1]

[1]Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Jammu and Kashmir, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SC wrote the first draft of the manuscript, wrote the protocol and performed the experiments. Author BAF designed the study, wrote the protocol, supervised the experimental work and wrote the manuscript. Author GB helped in writing protocol. Author AK helped in performing the experiments. Author MM managed the literature searches. Author MY managed the analyses of the study, performed the statistical analysis and helped in writing the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2017/36876
Editor(s):
(1) Kalpy Julien Coulibaly, Département Environnement et Santé (Intérim), Institut Pasteur de Côte d'Ivoire, Université Félix Houphouet-Boigny, Abidjan, Côte d'Ivoire.
Reviewers:
(1) Wagih Mommtaz Ghannam, Mansoura University, Egypt.
(2) Magda Ramadan Abdelwadood, Ain Shams University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/22237

Original Research Article

Received 21st September 2017
Accepted 20th October 2017
Published 11th December 2017

ABSTRACT

Both molecular and non-molecular tests currently employed for the detection of carbapenemases are costly, time-consuming and are poorly adapted to the clinical need for isolating patients rapidly to prevent health care associated outbreaks. The present study was thus conducted for rapid detection of carbapenemases in clinical isolates of Gram negative bacteria by Carba NP test. A total of 270 Gram-negative bacilli included were divided into three groups Group I (n=100): Gram negative bacteria isolated from routine samples. Group II (n=130): Imipenem resistant Gram negative bacteria. Group III (n=40): Twenty PCR confirmed for bla VIM and bla NDM1 and twenty PCR negative for bla VIM and bla NDM1 isolates. These isolates were subjected to Carba NP test. Antimicrobial susceptibility was performed by Kirby Bauer disk diffusion method. Out of 100 isolate (Group I), 32 were positive and out of 130 imipenem resistant (Group II), 112 isolates were positive for carbapenemase production by carba NP test. The sensitivity and specificity was 100% for

**Corresponding author: E-mail: bashirfomda@gmail.com;*

Carba NP test. This test is rapid, user-friendly and does not require costly equipment hence is suitable for resource poor settings. Rapid detection of carbapenemase by this test and timely institution of infection control measures will help in preventing spread of infection.

Keywords: Carba NP test; carbapenemase; gram negative bacteria; antibiotics; resistance.

1. INTRODUCTION

Overuse of antimicrobial agents and non-compliance of infection control practices have led to an increased incidence in infections due to multidrug resistant Gram negative bacteria. Carbapenems are often used as last resort antimicrobials to treat infections due to extended spectrum β-lactamase or plasmid mediated Amp C (Amp C) producing organisms. Unfortunately, the prevalence of carbapenem resistant *Enterobacteriaceae* (CRE), and Pseudomonas spp. has increased during the past 10 years and has seriously compromised the therapeutic armamentarium [1-3]. Moreover, Acinetobacter which was previously recorded as colonizer has emerged as an important pathogen in nosocomial infection, we have previously reported high frequency of carbapenem resistance in Acinetobacter from Kashmir with elaboration of blaOXA-23 and blaOXA-51. In another study from India emergence of OXA-51 in clinical strains of A. baumannii has been reported [4,5].

Phenotype based technique for identifying in-vitro production of carbapenemases, such as the modified Hodge test, has been used for years, but it is not highly sensitive and specific and is also time consuming [6-8]. Detection of imipenemase (IMP), Verona integrin metallo-β-lactamase (VIM), New- Delhi metallo-β-lactamase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC) producers may be based on the inhibitory properties of several molecules like tazobactam, clavulanic acid or boronic acid, but requires additional expertise and time (usually an extra 24 to 48 hours) and have variable sensitivity and specificity [6,7,9,10]. Furthermore, no inhibitors are available for detecting Oxacillinase (OXA-48) type producers that are spreading rapidly [6].

Detection of carbapenemase activity can be done using a UV spectrophotometer, which is available in many microbiology laboratories. This spectrophotometry based technique has 100% sensitivity and 98.5% specificity for detecting any kind of carbapenemase activity [11]. Although this technique can be implemented in any reference laboratory, it is costly and requires time. Recently, however the use of mass spectrometry for detection of carbapenemase activity has been proposed based on the analysis of the degradation of a carbapenem molecule [12,13]. Although this technique has to be further evaluated, matrix-assisted laser desorption ionization time-of- flight mass spectrometry (MALDI-TOF) equipment is increasingly being used in various reference diagnostic microbiology laboratories [7]. However, the cost of equipment hampers its use in routine diagnosis.

Molecular techniques remain the gold standard for the precise identification of organisms carrying the carbapenemase genes [7]. Most of these techniques are PCR based and may be followed by a sequencing step if a precise identification of the carbapenemase gene is needed [14-15]. PCR performed directly on colonies can give results within 4-6 h (or less when using real- time PCR technology) with excellent sensitivity and specificity. Similarly, other molecular techniques like microarray are useful for this purpose [16]. The main disadvantages of the molecular based technologies are their cost, the requirement of trained microbiologists and the inability to detect novel unidentified genes. Both molecular and non- molecular tests currently employed for the detection of genes encoding for carbapenemases are time-consuming (at least 12-24 hours) and are poorly adapted to the clinical need for isolating patients rapidly to prevent nosocomial outbreaks.

The most important and recent development for the accurate identification of carbapenemase is the Carba-NP test. It detects not only all known carbapenemases in Gram negative bacteria but has the potential to identify virtually any new emerging carbapenemases, in contrast to molecular techniques. The Carba-NP test is, user-friendly and inexpensive. In addition, it does not require any specific equipment and thus is suitable for resource poor settings.

In most of the studies Carba NP test was evaluated on isolates previously confirmed to be

carbapenemase producers by different methods [7,17]. The present study was conducted to rapidly detect carbapenemase producing Gram negative bacteria in clinical isolates by Carba NP test so as to prevent their dissemination in the environment by a timely institution of infection control measures.

2. MATERIALS AND METHODS

2.1 Study Period

This prospective study was conducted in the department of Microbiology, Sher-i- Kashmir Institute of Medical Sciences (SKIMS), a 660-bedded tertiary care institute over a period of one year (November 2013 to October 2014).

2.2 Clinical Isolates

Gram negative bacilli isolated from various clinical samples (blood, pus, urine, sputum and other body fluids) of patients admitted at Sher-i-Kashmir Institute of Medical Sciences was included in the study and identified using standard microbiological techniques. Repeated samples from the same patient and polymicrobial infection were excluded from the study.

The isolate recovered from various clinical samples were divided into the following groups. *Group I* (n=100): Gram negative bacteria isolated from routine samples. *Group II* (n=130): Imipenem resistant isolates from routine samples were also included. *Group III* (n=40): We also included twenty isolates previously confirmed by PCR for one of the following genes: *bla* NDM 1 (06) comprising of *E. coli* (04), *Klebsiella pneumoniae* (02) and, *bla* VIM (14) consisting of *P. aeruginosa* (08) and *Acinetobacter baumanii* (06). Twenty isolates which were negative for *bla* NDM1 (07) comprising of *E. coli* (03), *Klebsiella pneumoniae* (04) and, *bla* VIM (13) consisting of *P.aeruginosa* (11) and *Acinetobacter baumanii* (02).

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was performed by Kirby-bauer disc diffusion method according to CLSI guidelines [18]. The following antibiotic discs procured from Hi- Media Laboratories Pvt. Limited; Mumbai, India were used; ceftazidime (30 µg), ceftazidime-clavulanic acid (30/10 µg), cefoxitin (30 µg), ceftriaxone (30 µg), cefipime (30 µg), amikacin (30 µg), gentamicin (10 µg), tobramicin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), piperacillin-tazobactam (100/10 µg), ampicillin (10 µg) ampicillin-sulbactam (10/10 µg), ticarcillin-clavulanic acid (75/10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and polymyxin-B (300 units). In addition, nitrofurantoin (300 µg), norfloxacin (10 µg), were tested in urinary isolates. *E. coli* ATCC 25922 and *Pseudomonas* ATCC 27853 were used as controls for the disc diffusion test.

2.4 Detection of Carbapenemases by Carba NP Test

Rapid detection of carbapenemase was done by Carba NP test performed as per methods described by Nordmann et al. [7] with few modifications.

One calibrated loop (10 µL) of the isolate recovered from the sample was resuspended in a Tris-HCl 20 mmol/L lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific Pierce, Rockford, IL, USA), vortexed for 1 minute and incubated at room temperature for 30 minutes. The resulting bacterial suspension was centrifuged at 10,000 × *g* at room temperature for 5 minutes. Thirty microliter of the supernatant, corresponding to the enzymatic bacterial suspension, was mixed in a 96-well microtiter plate with 100 µL of a 1-mL solution made of 3 mg of imipenem monohydrate (Sigma Aldrich, Saint-Louis, Missouri, United States), pH 7.8, phenol red solution, and 0.1 mmol/L ZnSO4 (Sarabhai M chemicals, India). The phenol red solution was prepared by mixing 2 mL of a phenol red (BDH Lab Chemicals, Bombay, India) solution 0.5% (wt/vol) with 16.6 mL of distilled water. The pH value was then adjusted to 7.8 by adding drops of 1 N NaOH. A mixture of the phenol red solution and the enzymatic suspension being tested was incubated at 37°C for a maximum of 2 hours. *K. Pneumoniae* ATCC BAA-1705 was used as a positive control and E. *coli* ATCC 25922 was used as a negative control.

The Carba NP test was interpreted as follows:

(i) If the color of the wells containing imipenem plus ZnSO4 turned from red to yellow or orange the isolate was considered as carbapenemase producer.
(ii) If the color of the wells containing imipenem plus ZnSO4 did not turn red to yellow or orange the isolate was considered as non-carbapenemase producer (Fig. 1).

2.5 Ethical Clearance

The authors assert that all procedures contributing to this work comply with the ethical standards of the Indian Council of Medical Research guidelines on human experimentation and have been approved by the ethical clearance committee of the Sher-i-Kashmir Institute of Medical Sciences.

2.6 Statistical Analysis

Carba NP test was compared with PCR. Kappa test was employed to see the agreement between the two tests. Sensitivity and specificity of the test were calculated. All the results were discussed on 5% level of significance i.e. p-value less than 0.05 was considered significant.

3. RESULTS

Out of the 100 isolates (Group I), Carba NP test for carbapenemase production was positive in 32 isolates and 68 isolates were negative. Out of 130 imipenem resistant isolates (Group II) tested for carbapenemase production using Carba NP test, 112 isolates were positive for carbapenemase production, whereas 18 were negative. All PCR positive (20) isolates were positive by Carba NP test and PCR negative (20) were negative for carbapenemase production. Carba NP test displayed 100% sensitivity and specificity for detection of carbapenemases.

Patients from whom carbapenemase positive organisms (n=164) were isolated comprised of 96 males (58.5%) and 68 (41.5%) females, whereas patients from whom carbapenemase negative (n=86) isolates were recovered included 47 (54.6%) males and 39 (45.4%) females. The difference between the isolation of carbapenemase positive and negative isolates among males and females was not statistically significant (P>0.05). Most of the patients from whom carbapenemase positive organisms were isolated, were in the age group of ≥ 60 years, 39 (23.8%), followed by the age group of 50-59 years, 35 (21.3%); 20-29 years, 31 (18.9%); 40-49 years, 21 (12.8%); 30-39 years, 20 (12.2%); 10-19 years, 10 (6.1%); and 0-9 years, 8 (4.9%). On the other hand, patients from whom carbapenemase negative organisms were isolated, were in the age group of ≥ 60 years, 19 (22.1%) followed by the age group of 20-29 years, 18 (20.9%); 50-59 years, 17 (19.8%), 40-49 years, 16 (18.6%); 30-39 years, 12 (13.9%) 10-19 years, 3 (3.5%), 0-9 years, 1 (1.2%). There was no statistically significant difference between carbapenemase producers and non-producers among different age groups (P=0.367).

Maximum number of carbapenemase positive organisms were recovered from pus, followed by blood, swab, urine, sputum, Continuous ambulatory peritoneal dialysis (CAPD) fluid bile, drain fluid, pleural fluid, ascitic fluid and CSF (Fig. 2). However, no statistically significant association between type of samples and carbapenemase production was seen (P>0.05).

Carbapenemase positive isolates were isolated mostly from specimens obtained from patients in surgical intensive care unit (SICCU) 25 (15.2%), followed by gastroenterology and general medicine, 23 (14.0%) each; general surgery, 19 (11.6%); endocrinology 18 (10.9%); plastic surgery 15 (9.2%); nephrology 12 (7.3%); cardiovascular and thoracic surgery 10 (4.9%); medical oncology 8 (4.9%) neonatology 6 (3.7%), pediatric surgery 3 (1.8%) and cardiology 2 (1.2%). A significant number of carbapenemase positive isolates were from SICCU (P=0.013)

Majority of the carbapenemase positive organisms were *Acinetobacter spp*, 40 (24.4%) followed by *E. coli* and *K. pneumoniae* 37 (22.6%) each, *P. aeruginosa* 31 (18.9%), *E. cloacae* 11 (6.7%), *Citrobacter spp* 5 (3.0%) and *Proteus mirabilis* 3 (1.8%). A significantly higher number of *Acinetobacter spp* were found to be carbapenemase positive by Carba NP test. (P=0.009).

The antibiogram of the carbapenemase producing organisms (n=164) is shown in Table 1. Out of 164 isolates, 164 (100%) were resistant to imipenem followed by ceftriaxone, ciprofloxacin, ceftazidime, cefipime, amikacin, piperacillin + tazobactam, gentamicin, levofloxacin, and polymyxin B, in 145 (88.4%), 142 (86.6%), 140 (85.4%), 90 (54.9%), 86 (52.4%), 82 (50%), 69 (42.1%), 68 (41.5%) and 3 (1.8%) isolates respectively.

Out of a total of 96 isolates ampicillin+sulbactam resistance was seen in 75 (78.1%) isolates. Ticarcillin+clavulanic acid resistance was seen in 94 (74%) isolates out of a total of 127 isolates for which the antibiotic was tested. Co-trimoxazole resistance was seen in 87 (65.4%) isolates out of a total of 133 Gram negative bacteria. A total of 12 (38.7%) isolates were resistant to tobramycin and 10 (32.3%) to carbenicillin out of a total of 31

Pseudomonas isolates for which these antibiotics were tested. For urinary isolates (n=15) nitrofurantoin resistance was seen in 3 (20%) and norfloxacin resistance in 13 (86.7%) isolates.

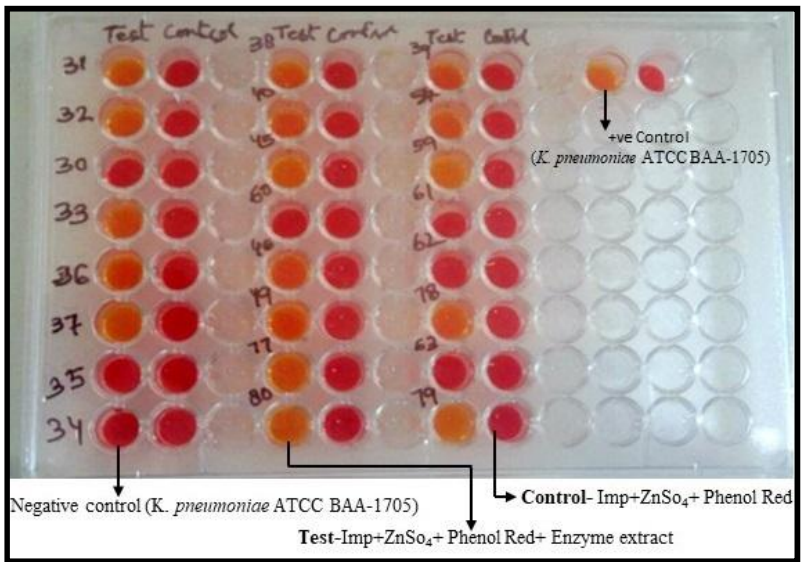

Fig. 1. Representing results of Carba NP test, showing carba NP positive and negative strains

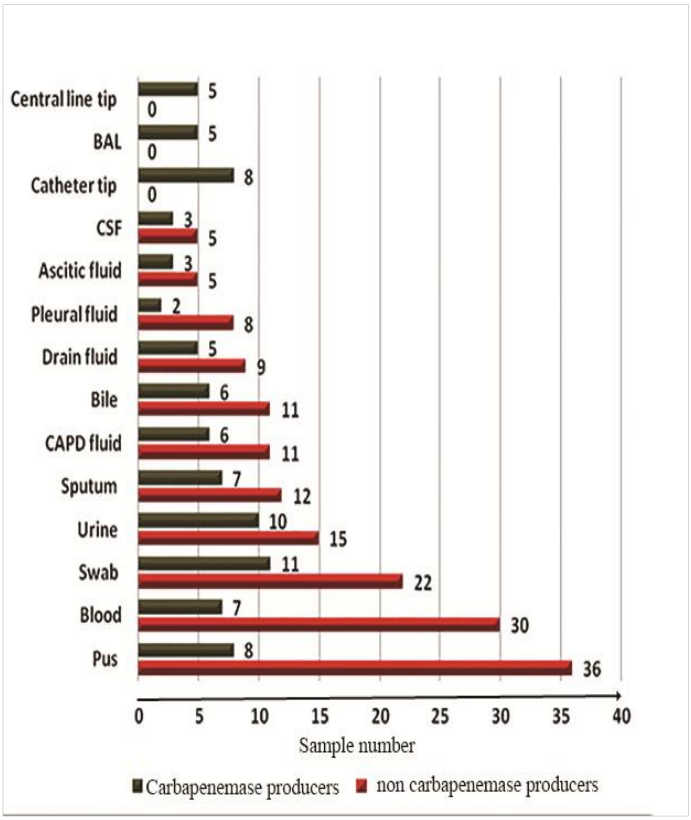

Fig. 2. Bar diagram showing sample wise breakup of carbapenemase +ve and -ve isolates by Carba NP test

Table 1. Antibiotic susceptibility profile of Carba NP test positive and negative isolates

Antibiotic	No tested	Carbapenemase positive		No tested	Carbapenemase negative		p-value
		S	R		S	R	
		N (%)	N (%)		N (%)	N (%)	
Amikacin	164	78 (47.6)	86 (52.4)	86	76 (88.4)	10 (11.6)	<0.001
Gentamicin	164	96 (58.5)	68 (41.5)	86	70 (81.4)	16 (18.6)	<0.001
Tobramycin	31	19 (61.3)	12 (38.7)	13	6 (46.2)	7 (53.8)	0.355
Carbenicillin	31	21 (67.7)	10 (32.3)	13	2 (15.4)	11 (84.6)	0.022
Ceftazidime	164	24 (14.6)	140 (85.4)	86	43 (50)	43 (50)	<0.001
Ceftriaxone	164	19 (11.6)	145 (88.4)	86	49 (57)	37 (43)	<0.001
Cefipime	164	74 (45.1)	90 (54.9)	86	47 (54.7)	39 (45.3)	0.152
Ampicillin +Sulbactam	96	21 (21.9)	75 (78.1)	56	21 (37.5)	35 (62.5)	0.038
Ticarcillin +Clavulanic acid	127	33 (26)	94 (74)	69	28 (40.6)	41 (59.4)	0.035
Piperacillin +Tazobactam	164	82 (50)	82 (50)	86	34 (39.5)	52 (60.5)	0.115
Ciprofloxacin	164	22 (13.4)	142 (86.6)	86	52 (60.5)	34 (39.5)	<0.001
Levofloxacin	164	95 (57.9)	69 (42.1)	86	37 (43.0)	49 (57)	0.025
Co-trimoxazole	133	46 (34.6)	87 (65.4)	73	48 (65.8)	25 (34.2)	<0.001
Nitrofurantoin	15	12 (80)	3 (20)	10	8 (80)	2 (20)	1.00
Norfloxacin	15	2 (13.3)	13 (86.7)	10	8 (80)	2 (20)	0.002
Imipenem	164	0	164 (100)	86	37 (43.0)	49 (57)	<0.001
Polymyxin B	164	161(98.2)	3 (1.8)	86	80 (93.0)	6 (7)	0.067

Carbapenemase negative isolates (n=86) also depicted variable sensitivity to the antibiotics tested. Out of 86 isolates 52 (60.5%) piperacillin + tazobactam followed by levofloxacin, imipenem, ceftazidime, cefipime, ceftriaxone, ciprofloxacin, gentamicin, amikacin, polymyxin B, in 49 (57%), 49 (57%), 43 (50%), 39 (45.3%), 37 (43%), 34 (39.5%), 16 (18.6%), 10 (11.6%) and 6 (7%) isolates respectively. Out of a total of 56 Isolates ampicillin+sulbactam resistance was seen in 35 (62.5%) isolates. Ticarcillin+clavulanic acid resistance was seen in 41 (59.4%) isolates out of a total of 69 isolates for which the antibiotic was tested. Co-trimoxazole resistance was seen in 25 (34.2%) isolates out of a total of 73 Gram negative bacteria. A total of 7 (53.8%) isolates were resistant to tobramycin and 11 (84.6%) to carbenicillin out of a total of 13 Pseudomonas isolates for which these antibiotics were tested. For urinary isolates (n=10) nitrofurantoin resistance was seen in 2 (20%) and norfloxacin resistance in 2 (20%) isolates.

Higher resistance pattern of carbapenemase positive isolates was seen to the various antibiotics tested with statistically significant values seen for amikacin, gentamicin, ceftazidime, ceftriaxone, ciprofloxacin, co-trimoxazole, imipenem ($P<0.001$) and carbenicillin ($P=0.022$), ampicillin + sulbactam ($P=0.038$), ticarcillin + clavulanic acid ($P=0.035$), levofloxacin ($P=0.025$) and norfloxacin ($P=0.002$).

4. DISCUSSION

Multidrug resistance among a variety of bacterial species is a worldwide phenomenon causing both community acquired and health care associated infections. One of the most important resistant traits corresponds to the production of the carbapenem hydrolysing beta-lactamases that confer resistance to almost all β-lactams [19]. Therapeutic options for the management of infections due to carbapenemase producing bacteria are limited and morbidity and mortality is high. Resistance due to carbapenemase production has a potential for rapid dissemination as it is often plasmid mediated. Consequently, timely detection of these plasmid borne and easily transmissible carbapenemases is important given the implications for infection control [17].

Various phenotypic methods viz; modified Hodge test (MHT), double disc synergy test (DDST), combined disc synergy test (CDST), MBL E-test are used to detect the presence of carbapenemases in clinical isolates [20]. However, these tests suffer from the lack of specificity, a long turnaround time and poor sensitivity to detect metallo-beta- lactamase production (especially for MHT). Also, their utility in detecting carbapenemase in non-fermenters is limited. Molecular methods on the other hand although being sensitive and specific are of limited practical use for daily application in clinical laboratories due to their high cost [21].

Thus a simple, rapid and inexpensive method to detect carbapenemases in clinical isolates is necessary for targeted therapy and more importantly to limit the spread of bacteria producing these enzymes.

The Carba NP test is a novel phenotypic method developed for carbapenemase detection in Gram negative bacteria that is based on the in vitro hydrolysis of imipenem by a bacteria lysate and is detected by changes in pH values using the indicator phenol red. The present study was thus undertaken to rapidly detect the presence of carbapenemases in clinical isolates of Gram negative bacteria by Carba NP test in our hospital.

Out of the 100 GNB comprising Group I, Carba NP test, was positive in 32 isolates and negative in 68 isolates. Thus, 32% of isolates were carbapenemase producers. In this group as we included all Gram-negative organisms irrespective of imipenem sensitivity so only 32 isolates were found to be carbapenemase positive. Kali et al. [22] reported a prevalence of 22.4% in their study. High prevalence of carbapenemases producers in our study are the cause of concern.

Group II comprised of 130 isolates recovered from clinical specimens that were resistant to imipenem on the disc diffusion test. High prevalence of carbapenemase seen in Group II was expected as we included isolates which were resistant to imipenem. In this group mechanisms, other than carbapenemase production like loss of porin channels or increased efflux pump activity can be a reason for the 18 imipenem resistant isolates that were negative by Carba NP test.

In the present study Carba NP test was found to be 100% sensitive and specific when compared to PCR for detection of carbapenemase similar observation were made by Nordman P, et al. [19], who developed the Carba NP test for the rapid detection of the carbapenemase enzyme in

clinical isolates of *Enterobacteriaceae*. The authors concluded that the Carba NP test perfectly differentiates carbapenemase producers from strains that are carbapenem resistant due to non-carbapenemase mediated mechanism. On the other hand, Tijet N, et al. [23], in their study on the evaluation of the Carba NP test for rapid production of carbapenemase producing *Enterobacteriaceae* and *P. aeruginosa*, reported 100% specificity and 80% sensitivity.

Although Carba NP test has consistently been found to be 100% specific for the detection of carbapenemase producing *Enterobacteriaceae,* its sensitivity has been shown to range from 72.5 to 100% [24]. In the recent study RAPIDDEC CARBA NP showed 99% sensitivity and 100% specificity of for detection of carbapenemase [25].

In a comparison of the Carba NP test with the modified Hodge test, for detecting carbapenemase producing Gram negative bacilli, the two tests were found to be equally sensitive with the Carba NP test exhibiting higher specificity (100% as compared to MHT (80%) [20]. Likewise, Huang TD et al. [26] compared the Carba NP test to another chromogenic based assay, the Rosco Rapid CARB screen and reported a better performance of the Carba NP test, owing to its superior specificity. The Carba NP test has been evaluated to detect carbapenemase producing *Pseudomonas spp*. directly from blood cultures [17]. It was found that the Carba NP test performed directly from spiked blood cultures, perfectly differentiated carbapenemase producers from carbapenem resistant isolates with non carbapenemase mediated mechanisms. In their study on the phenotypic detection of carbapenemase producing *Enterobaceriaceae* by the use of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry and the Carba NP test Knox J et al. [24], found that although MALDI-TOF MS performed well, overall the easy to use phenotypic Carba NP test makes it ideal for use in routine laboratory settings. Moreover, Carba NP test has been recently included in CLSI guidelines as a confirmatory test for detection of carbapenemases in Gram negative bacteria [27].

No significant difference in the isolation of carbapenemase positive and negative isolates among males and females was seen (P>0.05). Isolation of carbapenemase positive and negative isolates among different age groups was also not statistically significant (P=0.367) even though majority of the carbapenemase positive and negative GNB were isolated from patients in the age group of ≥ 60 years. Similarly, Hirakata et al found no age or sex related difference between MBL producers and non-producers. This finding was also corroborated by Zavascki et al. [28,29].

No statistically significant association between type of samples and carbapenemase positive and negative results was seen (P>0.05). This is in contrast with another study by Hirakata et al in which predominant source was urine which was attributed to the use of indwelling urinary catheter [28].

Significant isolation of carbapenemase producing isolates was seen from SICCU (P=0.013). A similar observation was made by Zavascki et al. [29] who found ICU stay as a risk factor for acquisition of MBL producing *Pseudomonas aeruginosa*.

In present study, a significantly higher number of *Acinetobacter* spp were found to be carbapenemase producer 40 (24.4%); (P=0.009). Infections caused by this organism have been reported globally and are increasing in incidence these days and since majority of *A. baumannii* recovered from patients are MDR, treatment of these infections is challenging [30,31].

Our study indicates carbapenemase producing isolates were resistant to several antibiotic classes. This has been validated in several other studies [32-34]. High proportion of isolates which were carbapenemase producers were susceptible to polymixin B [35]. Polymyxin B and colistin remains the mainstay of treatment for multi-drug resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The alternative therapeutic strategies for MDR *Acinetobacter baumannii* include the use of rifampicin, travofloxcin, doxycycline, minocycline or tigecycline with sulbactam [36].

Carbapenemase producing isolates are a cause of great clinical concern as they are associated with resistance to many classes of potent antimicrobial agents including aminoglycosides, β-lactams, fluoroquinolones and carbapenems. Acquisitions of multidrug resistant Gram negative bacteria are related to environmental contamination and contact with transiently colonized healthcare providers. Control measures addressing these sources of infection

have been proved to be successful in controlling the spread of these organisms. Hence continued careful attention to hand hygiene, contact isolation, barrier precautions, adequate environmental cleaning and careful disinfection of patient care equipments along with surveillance are essential to prevent outbreak of infections caused by multidrug resistant strains [36].

5. CONCLUSION

Rapid detection of carbapenemases in clinical isolates of Gram negative bacteria by Carba NP test will help in preventing the spread of infection caused by these organisms by timely institution of infection control measures. This test is rapid, user-friendly and does not require any specific equipment and thus is suitable for resource poor settings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Livermore DM, Woodford N. The beta-lactamase threat in enterobacteriaceae, *Pseudomonas* and *Acinetobacter*. Trends in Microbiology. 2006;14:413-20.
2. Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, others. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clinical Microbiology and Infection. 2012;18:413-431.
3. Casellas JM. Antibacterial drug resistance in Latin America: Consequences for infectious disease control. Revista panamericana de Salud Publica = Pan American Journal of Public Health. 2011; 30:519-28.
4. Bali NK, Fomda BA, Bashir H, Zahoor D, Lone S, Koul PA. Emergence of carbapenem-resistant *Acinetobacter* in a temperate north Indian State. British Journal of Biomedical Science. 2013;70: 156-60.
5. Tiwari V, Kapil A, Moganty RR. Carbapenem-hydrolyzing oxacillinase in high resistant strains of *Acinetobacter* baumannii isolated from India. Microbial Pathogenesis. 2012;53:81-86.
6. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing enterobacteriaceae. Emerging Infectious Diseases. 2011;17:1791-8.
7. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing enterobacteriaceae. Emerging Infectious Diseases. 2012;18:1503-7.
8. Pasteran F, Mendez T, Rapoport M, Guerriero L, Corso A. Controlling false-positive results obtained with the Hodge and Masuda assays for detection of class a carbapenemase in species of enterobacteriaceae by incorporating boronic acid. Journal of Clinical Microbiology. 2010;48:1323-32.
9. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. Characterization of a new metallo- -lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrobial Agents and Chemotherapy. 2009;53:5046-5054.
10. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. Journal of Clinical Microbiology. 2011;49:718-21.
11. Bernabeu S, Poirel L, Nordmann P. Spectrophotometry-based detection of carbapenemase producers among Enterobacteriaceae. Diagnostic Microbiology and Infectious Disease. 2012; 74:88-90.
12. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. Journal of Clinical Microbiology. 2011;49:3321-3324.
13. Hrabak J, Walkova R, Studentova V, Chudackova E, Bergerova T. Carbapenemase activity detection by matrix-assisted laser desorption ionization-

time of flight mass spectrometry. Journal of Clinical Microbiology. 2011;49:3222-7.

14. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic Microbiology and Infectious Disease. 2011; 70:119-123.
15. Avlami A, Bekris S, Ganteris G, Kraniotaki E, Malamou-Lada E, Orfanidou M, Paniara O, Pantazatou A, Papagiannitsis CC, Platsouka E, a others. Detection of metallo-β-lactamase genes in clinical specimens by a commercial multiplex PCR system. Journal of Microbiological Methods. 2010;83:185-187.
16. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray for the rapid detection of extended-spectrum beta-lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). The Journal of Antimicrobial Chemotherapy. 2012;67: 1865-9.
17. Dortet L, Poirel L, Errera C, Nordmann P. CarbAcineto NP test for rapid detection of carbapenemase-producing *Acinetobacter* spp. Journal of Clinical Microbiology. 2014; 52:2359-2364.
18. Wayne PA. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: 23th Informational Supplement. Clinical and Laboratory Standards: M100-S23; 2013.
19. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V, European Network on C. Identification and screening of carbapenemase-producing Enterobacteriaceae. Clinical microbiology and infection: The official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012;18:432-8.
20. Vasoo S, Cunningham SA, Kohner PC, Simner PJ, Mandrekar JN, Lolans K, Hayden MK, Patel R. Comparison of a novel, rapid chromogenic biochemical assay, the carba NP test, with the modified hodge test for detection of carbapenemase-producing gram-negative bacilli. Journal of Clinical Microbiology. 2013;51:3097-3101.
21. Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. The Indian Journal of Medical Research. 2009;129:707-12.
22. Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A, Umadevi S. Detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa* in intensive care units. The Australasian Medical Journal. 2013;6:686-693.
23. Tijet N, Boyd D, Patel SN, Mulvey MR, Melano RG. Evaluation of the carba NP test for rapid detection of carbapenemase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2013;57: 4578-4580.
24. Knox J, Jadhav S, Sevior D, Agyekum A, Whipp M, Waring L, Iredell J, Palombo E. Phenotypic detection of carbapenemase-producing Enterobacteriaceae by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry and the Carba NP test. Journal of Clinical Microbiology. 2014;52:4075-7.
25. Aktas E, Malkocoglu G, Otlu B, Copur Cicek A, Kulah C, Comert F, Sandalli C, Gursoy NC, Erdemir D, Bulut ME. Evaluation of the carbapenem inactivation method for detection of carbapenemase-producing gram-negative bacteria in comparison with the RAPIDEC CARBA NP. Microbial Drug Resistance (Larchmont, NY); 2016.
26. Huang TD, Berhin C, Bogaerts P, Glupczynski Y. Comparative evaluation of two chromogenic tests for rapid detection of carbapenemase in *Enterobacteriaceae* and in *Pseudomonas aeruginosa* isolates. Journal of Clinical Microbiology. 2014;52: 3060-3.
27. Wayne PA. CLSI. Performance standards for antimicrobial susceptibility testing: 25th; 2015.
28. Hirakata Y, Yamaguchi T, Nakano M, Izumikawa K, Mine M, Aoki S, Kondoh A, Matsuda J, Hirayama M, Yanagihara K, others. Clinical and bacteriological characteristics of IMP-type metallo-beta-lactamase-producing *Pseudomonas aeruginosa*. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. 2003;37:26-32.
29. Zavascki AP, Barth AL, Gaspareto PB, Goncalves AL, Moro AL, Fernandes JF, Goldani LZ. Risk factors for nosocomial infections due to *Pseudomonas*

aeruginosa producing metallo-beta-lactamase in two tertiary-care teaching hospitals. The Journal of Antimicrobial Chemotherapy. 2006;58:882-5.

30. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrobial Agents and Chemotherapy. 2007;51:3471-3484.
31. Landman D, Bratu S, Kochar S, Panwar M, Trehan M, Doymaz M, Quale J. Evolution of antimicrobial resistance among *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* in Brooklyn, NY. The Journal of Antimicrobial Chemotherapy. 2007;60:78-82.
32. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo--lactamases: the quiet before the storm? Clinical Microbiology Reviews. 2005;18:306-325.
33. Peleg AY, Franklin C, Bell JM, Spelman DW. Dissemination of the metallo--lactamase gene blaIMP-4 among gram-negative pathogens in a clinical setting in Australia. Clinical Infectious Diseases. 2005;41:1549-1556.
34. Cornaglia G, Mazzariol A, Lauretti L, Rossolini GM, Fontana R. Hospital outbreak of carbapenem-resistant pseudomonas aeruginosa producing VIM-1, a novel transferable metallo--lactamase. Clinical Infectious Diseases. 2000;31:1119-1125.
35. Fomda BA, Khan A, Zahoor D. NDM-1 (New Delhi metallo beta lactamase-1) producing gram-negative bacilli: Emergence & clinical implications. The Indian Journal of Medical Research. 2014;140:672-678.
36. Shanthi M, Sekar U. Multi-drug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections among hospitalized patients: Risk factors and outcomes. The Journal of the Association of Physicians of India. 2009;57:636,638-40,645.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22237