Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/5233
DOI URL: http://dx.doi.org/10.21474/IJAR01/5233

RESEARCH ARTICLE

PERFORMANCE OF RAPIDEC CARBA NP TEST IN DETECTION OF CARBAPENEMASE PRODUCING GRAM-NEGATIVE BACTERIA.

Soheir Abd El Rahman[1], MD, Yasser Mahmoud Ismail[1], MD, Afaf Fathi Khamis[1], and MD. Huda Al-shahat Mohamed[2].

1. Clinical and Chemical Pathology Department, Faculty of Medicine, Benha University.
2. *M.B.B.ch* Faculty of Medicine, Benha University.

Manuscript Info

Manuscript History

Received: 21 June 2017
Final Accepted: 23 July 2017
Published: August 2017

Key words:-
Carbapenem resistance, Carbapenemase, Phenotypic methods, genotypic analysis, Carba NP test.

Abstract

Objective: The objective of this study is to assess the performance of Rapidec carba NP test in the reliable detection of carbapeneme resistance in Gram negative bacteria.

Methods: This study was performed at the Microbiology unit, Clinical Pathology Department at Benha University Hospital. A total of 60 stored isolates were included in the study. These isolates were Gram negative bacilli and were characterized phenotypically and genotypically for the presence of carbapenemases. Fifty isolates were confirmed to be carbapenemase + ve and they were used as study group. Ten isolates were confirmed to be carbapenemase - ve and were used as negative control. Rapidec carba NP test was applied according to manufacturer's instructions and results were read after 30 and 120 minutes of incubation.

Results: Ready-to-use Rapidec carba NP gave 100% sensitivity, specificity positive predictive value and negative predictive value when manufacturer's instructions were followed. This was in accordance with PCR results. Rapid detection of carbapenemases genes is of great importance, since these MDR organisms have the potential to spread rapidly in hospital environments and cause nosocomial infections with high mortality rates. Molecular methods for detection of carbapenem resistance are the gold standard, however they are expensive, time consuming and require specialized equipment and experience.

Conclusion: Rapidec carba NP test is rapid, easy to perform and interpret, relatively inexpensive and present a practical solution for rapid detection of carbapenem resistant gram negative bacteria.

Introduction:-

Carbapenems are often used as "antibiotic of last resort" when patients with infection became gravely ill or suspected of harboring resistant bacteria. Carbapenem resistance in Enterobacteriaceae, Pseudomonas, *Acinetobacter baumannii* and other bacteria is now emerging worldwide at an alarming rate, causing both nosocomial and now community-acquired infections [(1)].

Corresponding Author:- Soheir Abd El Rahman.
Address:- Clinical and Chemical Pathology Department, Faculty of Medicine, Benha University.

Carbapenemases are β-lactamases that are capable of inactivating most existing β-lactams. Their spread among gram-negative bacilli is a major public health concern. A variety of carbapenemases have been reported in these bacteria such as KPC (Ambler class A), metallo-β-lactamases of VIM-, IMP- and NDM-type (Ambler class B), and OXA-48-types (Ambler class D). Thus, an efficient strategy for detection of carbapenemase producers is becoming critical for the determination of appropriate therapeutic schemes and the implementation of infection control measures [(2)].

Recently, the Carba NP test has been developed for rapid identification of carbapenemase production in Enterobacteriaceae. Here, we further improve and evaluate the ability of the Carba NP test to detect carbapenemase producers among Enterobacteriaceae recovered from various commercial media (selective, non-selective and screening media) used in clinical situations.

Materials and Methods:-

Sample:-

This study was performed at the Microbiology unit, Clinical Pathology Department at Benha University Hospital. A total of 60 stored isolates on broth that had been collected from ICU of Benha University Hospital were included in the study. The isolates were Gram negative bacilli that were characterized phenotypically and genotypically for the presence of carbapenemases. Fifty isolates were confirmed to be carbapenemase + ve and were used as study group. Ten isolates were confirmed to be carbapenemase - ve and were used as negative control.

Methods:-

Test for viability (subculture of the isolates):-

The stored isolates were incubated at 37^{o}c until they had been dissolved. They were tested for viability by subculturing on selective media (MacConkey agar) and enrichment non-selective media (blood agar).

Identification:-

Isolates were identified by conventional methods such as culture characteristics and biochemical reactions. Oxidase test, Triple sugar iron agar (TSI), Lysine iron agar (LIA), media based on motility, ornithine decarboxylase activity, and indole production (MIO), Simmons citrate agar and Urea agar base (Oxoid Co. England) were used.

Detection of carbapenem resistance:-

a) Disk diffusion method:-

Disk diffusion susceptibility testing was performed according to CLSI 2016 recommendations. Antibiotic disks and Muller Hinton agar were obtained from Becton Dickenson (Franklin Lakes, NJ). Zone diameters were recorded. Results were categorized as sensitive, intermediate and resistant.

b) Genotypic Real time PCR analysis:-

Genomic DNA Extraction from isolated colonies

Well-isolated colonies were used for DNA extraction using a Genomic DNA purification kits (Tiagen, UK) according to the protocol suggested by the manufacturer. Briefly, a bacterial suspension equivalent to that of a 2 McFarland standard was prepared in saline, and bacterial DNA was extracted from a 200-µl (1.2 × 108 CFU) suspension. Extracted bacterial DNA was eluted from the columns in 200 µl elution buffer and stored at −20°C.

DNA extraction:-

1. Bacterial suspension was centrifuged for 1 min at 14.000 rpm. The pellet was taken and added to 20µl of Proteinase K solution, mixed by vortexing.
2. Lysis solution (200µl) was added and mixed thoroughly by vortexing to obtain a uniform suspension.
3. The sample was incubated at 56 °C for 20 minutes in shaking water bath until the cells were completely lysed.
4. Ethanol 80% (250µl) was added and mixed by pipetting.
5. The prepared mixture was transferred to the spin column and centrifuged for 1 minute at 6,000 Xg.
6. Wash buffer I (500 µl) was added then centrifuged for 1 minute at 8,000 Xg .
7. Wash buffer II (500µl) was added to the column and centrifuged for 3 minute at maximum speed.
8. Elution buffer (200 µl) was added to the center of the column membrane to elute genomic DNA and incubated for 2 minute at room temperature then centrifuged for 1 minute at 8,000 xg.
9. The purification column was discarded and the purified DNA used immediately in downstream applications or stored at -20 °C.

Amplification using Multiplex real-time PCR:-

The details of the reference genes used in this assay were obtained from previous studies. These genes were: class A carbapenemases encoding GES and KPC type, class D oxacillinases encoding OXA-48 and class B metallo enzymes encoding NDM, IMP and VIM ***(Dallenne et al., 2010).***Amplification was performed using the following primer sets provided by (Operon, inc Huntsville, Alabama Germany). KPC-F:5'- TCGCTAAACTCGAACAGG-3'KPC-R: 5'TTACTGCCCGTTGACGCCCAATCC - 3'for blaKPC type, NDM-F: 5' TTGGCCTTGCTGTCCTTG-3'NDM-R:5' ACACCAGTGACAATATCACCG -3' for blaNDM type , GES-F primer 5'- CTATTACTGGCAGGGATCG-3', reverse primer 5'-CCTCTCAATGGTGTGGGT- 3' or blaGES type, OXA-48-F 5'-TGTTTTTGGTGGCATCGAT-3', OXA-48-R: 5'GTAAMRATGCTTGGTTCGC -3' for blaOXA-48 type, IMP-F: 5' GAGTGGCTTAATTCTCRATC-3' for blaIMP type and VIM-F :5' GTTTGGTCGCATATCGCAAC-3',VIM R:5'AATGCGCAGCACCAGGATAG-3'***(Dallenne et al., 2010).***

Amplifications were performed in 20 µl of the Master Mix reaction containing 10 µl PCR Master Mix, Syper Green dye (Tiagen, UK), a sufficient quantity of sterile water, primers and 2 mL of the DNA template. The pairs of primers were optimized to a final concentration of 1.2 mµ. The PCR runs were performed using the six positive controls and RNase-free water. The real-time PCR conditions were as follows: 95°C for 5 min. as initial denaturation then cycling for 35 cycle (95°C 30 Sec., (48-52°C) 45 sec and 72°C 1 min). The final extension was 72°C for 2 min. and a melt curve step (from 65 C gradually increasing by 0.1 C/s to 95 C using 7900HT fast real-time PCR system (Applied Biosystems). Analysis of amplified products was done by SDS software.

Procedure and detection:-

1. Syper Green PCR Master Mix , template DNA, primers and probe solutions, and RNase-free water were thawed. The individual solutions were mixed.
2. A reaction mix was prepared**.**
3- The samples were gently vortexed and spin down.
4- PCR was performed by using the recommended thermal cycling conditions outlined below.

The real-time PCR conditions:-

The real-time PCR conditions were as follows: 95^{o} C for 5 min; 35 cycles of 95^{o} C for 20 s, 55^{o} C for 45 s and 72 ^{o}C for 30 s; and a melt curve step (from 65^{o} C gradually increasing by 0.18^{o} c/s to 95^{o} C.

Each one of the six carbapenemase genes tested presented a different melting curve after PCR amplification.The melting temperature (Tm) analysis of the amplicons identified was as follows: blaIMP type (Tm 80.18^{o}c), blaOXA-48 (Tm 81.68 ^{o}C), blaNDM-1 (Tm 848 ^{o}C), blaGES type (Tm 88.68 ^{o}C), blaVIM type (Tm 90.38 ^{o}C) and blaKPC type (Tm 91.68 ^{o}C). No amplification was detected among the negative samples. The results showed 100% concordance with the genotypes previously identified.

RAPIDEC CARBA NP test:-

Principle: The test used in this study consists of a ready- to use strip and is based on the detection of carbapenem hydrolysis by carbapenemase-producing gram-negative bacteria ***(1)***. Hydrolysis acidifies the medium which results in the change in color of the pH indicator.

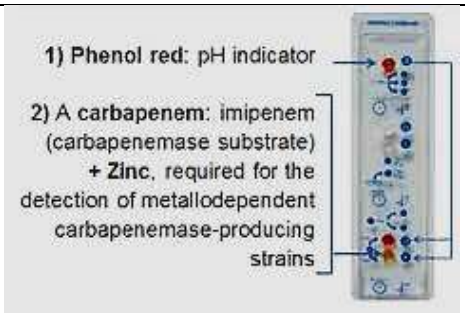
After bacterial lysis, which enables the extraction of the enzyme, the lysate is added to a detection solution containing:

- A carbapenem: Imipenem (carbapenemase substrate)
- Phenol red (pH indicator)
- Zinc, required for the detection of metallo-dependant carbapenemase-producing strains.

After incubating for a maximum of 2 hours, reading is performed visually by comparing a control well without imipenem to a reaction well containing imipenem ***(Nordmann&Poirel, 2013)***.

Composition of the test strip

Well	Reagent
A	Phenol red solution
B	Turbidity control
C	Lysis buffer
D	Control well without imipenem
E	Reaction well containing imipenem



Preparation of the test:-

1. The strip was removed from its packaging and the specimen reference number was written on the strip
2. One hundred microliter of API suspension medium was dispensed into each of the wells a, b and c.
3. A lid was placed on the strip and left for 4-10 minutes at room temperature (15-25^{o}C) and then the contents of well b were gently mixed using a stick. The strip was placed on the two colored (black and white) support to facilitate comparison of the contents in wells c
4. With the end of a new stick, several colonies of the same morphology were picked up and the contents of the stick were deposited in well c and were mixed until a turbidity equivalent to that of well b was obtained.
5. The strip was then left for 30 minutes at room temperature (15-25^{o}C)

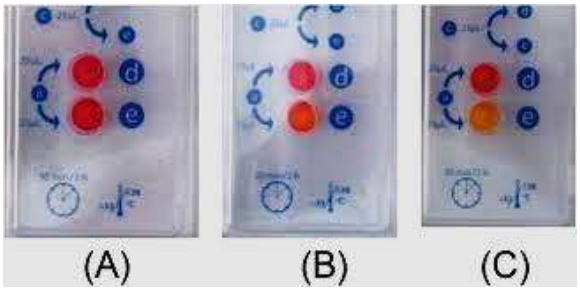
Procedure:-

Twenty-five microlitre from well c were transferred to wells d and e then 25 microliter from well "a" were transferred to wells "d" and "e" and then incubation at 33-38^{o}C was performed and we read after 40 minutes of incubation. In case of negative reactions, re-incubation for 1.5 hours was performed.

Reading and interpretation:-

Reading of the strip wells had been occurred as follow:

Interpretation	**Test well**	**Control well**
Negative	Red	Red
	Orange	Orange
Positive	Orange-Red, Orange to Yellow	Red
	Yellow	Orange
Non- interpretable	Not applicable	Any color other than red or orange
	Red	Orange



A) Negative, B) &C) Positive

Results:-

As regard the distribution of organisms in this study *Klebseilla Pneumoniae* were 30(60%) of test samples. Pseudomonas represented 16% (8 samples), Twelve samples were Enterobacter spp. (24 %), 3(6%) were *Proteus mirabilis*, Only one isolate was Acinetobacter spp. representing 2 %, One isolate was Serratia which represents 2% **(Table: 1)** .

Results of Carbapenem susceptibility testing are shown in **table (2, 3):-**

Genetic characterization of the isolates in the study group showed that out of *Klebseilla pneumoniae* 2(5.7%) isolate was positive for VIM, 15(43%) were positive for KPC, VIM, 8(22.9%) isolates were positive for KPC, VIM, OXA ,2(5.7%) isolate was VIM, NDM, 3(8.7%) isolates were positive for KPC, GES. Out of Pseudomonas spp. 4(50.0%) isolates were positive for KPC, VIM, 2(25%) isolates were positive for VIM, NDM, 2(25%) isolates were positive for VIM, IMP. Seven Enterobacter spp., (58.3%) isolates were positive for KPC, VIM, 5(41.3%`) were negative for. The only one isolate of Acinetobacter spp., 1(100%) was positive for VIM, IMP. Out of *Proteus mirabilis* 2(66.6%) isolate was positive for KPC, VIM, 1(33.3%) was positive for VIM. The isolate of Serratia 1(100%) isolate was positive for KPC, VIM, OXA. **(table 4).**

Rapidec carba NP gave 100% sensitivity, specificity, positive predictive value and negative predictive value when manufacturer's instructions were followed **(table5).** We found that there is excellent agreement between Rapidec carba NP test results and multiple PCR results **(table 6).**

Table (1):- Distribution of gram negative bacilli isolates regarding the species

Organism	**Test isolate**	**Control isolate**
Klebsiella pneumonia	30	5
Enterobacter	7	5
Pseudomonas	8	0
Proteus mirabilis	3	0
Acinetobacter	1	0
Serratia	1	0
Total	50	10

Table (2):- Relation between type of organism and carbapenems susceptibility results

,Organism		Imipenem			Total	Meropenem			Total	Ertapenem		Total
		R	I	S		R	I	S		R	S	
Klebsiella Pneumoniae	Count	30	0	5	35	26	4	5	35	30	5	35
Pseudomonas	Count	8	0	0	8	7	0	1	8	8	0	8
Enterobacter	Count	6	1	5	12	8	0	4	12	7	5	12
Proteus mirabilis	Count	3	0	0	3	3	0	0	3	3	0	3
Acinetobacter	Count	1	0	0	1	1	0	0	1	1	0	1
Serratia	Count	1	0	0	1	1	0	0	1	1	0	1
Total		49	1	10	60	46	4	10	60	50	10	60

This table shows that there is 30 Klebsiella isolates were resistant to imipenem and only 5 were susceptible. Pseudomonas species show that the overall 8 isolates were resistant to imipenem. For enterobacter 4 isolates were resistant to imipenem while one isolate showed intermediate susceptibility. Two isolates were sensitive. all Proteus isolates were resistant to imipenem. Acinetobacter isolate was resistant to imipenem. Also an isolate of Serratia showed imipenem resistance.

Table (3):- Distribution of different genes among different isolates:

		Organism						Total
		K. Pneumoniae	Pseudo-monas	Entero-bacter	*Proteus mirabilis*	Acineto-bacter	Serratia	
VIM	Count	2	0	0	2	0	0	4
	%	5.7%	.0%	.0%	66.7%	0.0%	0.0%	5.0%
KPC,VIM	Count	15	4	7	1	0	0	27
	%	43%	50.0%	58.3%	33.3%	0.0%	0.0%	45%
KPC,VIM,OXA	Count	8	0	0	0	0	1	9
	%	22.9%	.0%	.0%	.0%	0.0%	100%	12.5%
VIM,NDM	Count	2	2	0	0	0	0	4

	%	5.7%	25.0%	.0%	.0%	0.0%	0.0%	5.0%
KPC,GES	Count	3	0	0	0	0	0	3
	%	8.6%	.0%	.0%	.0%	0.0%	0.0%	5.0%
VIM,IMP	Count	0	2	0	0	1	0	3
	%	.0%	25.0%	.0%	.0%	100%	0.0%	7.5%
Total	Count	30	8	7	3	1	1	50

FET=38.9 P=0.13 (NS)

Table (4):- Rapidec Carba NP results

Rapidec Carba NP	No.	%
Negative	10	16.7
Positive	50	83.3
Total	60	100

Table (6):- Degree of agreement between PCR and carba NP results

			PCR results		Total
			-ve	+ve	
Carba NP2	Negative	Count	10	0	10
		% within PCR results	100%	0.0%	16.7%
	Positive	Count	0	50	50
		% within PCR results	0.0%	100%	83.3%
Total		Count	5	35	40
		% within PCR results	100%	100%	100%

Kappa test =1.0 **P**<0.001 (HS)

This table shows that all positive results for PCR are carba NP positive and all negative results for PCR are carba NP negative. According to value of K, there is excellent agreement between PCR results and carba NP results.

Discussion:-

Multidrug resistance is emerging worldwide at an alarming rate among a variety of bacterial species, causing both community-acquired and nosocomial infections. Carbapenems, the last line of treatment, are now frequently needed to treat nosocomial infections, and increasing resistance to this class of β-lactams represents a major concern in public health[(1)].

Carbapenemases are β-lactamases that are capable of inactivating most existing β-lactams. A variety of carbapenemases have been reported in gram-negative bacteria. Thus, an efficient strategy for detection of carbapenemase production has been critical for the determination of appropriate therapeutic schemes and the implementation of infection control measures [(2)].

Laboratory detection of carbapenemase producing bacteria is a challenge. Many strains have carbapenem MICs in the susceptible range, and different phenotypic methods such as the modified cloverleaf test and disk tests with different inhibitors, lack specificity and sensitivity. Detection of carbapenemase genes and the ability of a strain to hydrolyze carbapenems remain the gold standard methods of identification. Gene detection is, however, out of reach for many clinical laboratories. Therefore, the recently published Carba NP method has the potential to fill a gap [(7)].

Commercially available tests have several advantages over laboratory-developed assays, i.e., production is highly standardized and the quality is controlled by the manufacturer, reagents are preprepared and ready to use, and the shelf-life of the test kits is guaranteed [(8)].

In our study, a total of 60 Gram negative bacilli isolates were enrolled for evaluation of Rapidec carba. The isolates were collected from patients who were admitted in ICU at Benha university hospitals. Bacterial suspensions were stored in freezing tubes at – 20°c.The enrolled isolates were as follow, Klebsiellae spp. (58.3%), *Pseudomonas aeruginosa* (13%), Enterobacter spp. (12%), *Proteus mirabilis* (5%),Acinetobacter spp. (1.7%) and Serratia (1.7)

On the other hand, more or less different presentations of isolated bacteria were recorded all over the world. ***Yen Tan et al*** [10] found in the fourth quarter of 2007 that the isolated bacteria in Singapore hospitals were *E. Coli* (22%), *Staphylococcus aureus* (16%), Klebsiellae spp. (12%), *Pseudomonas aeruginosa*(9%), Enterococcus spp. (5%) and Acinetobacter spp. (3%).

Parveen et al. [11] stated that between May to July 2008, in a tertiary care hospital in South India, the distribution of pathogens among 235 consecutive Gram negative clinical isolates were *E. Coli* (27.65%), *Klebsiella pneumoniae* (16.17%), Acinetobacter spp. (5.1%), Citrobacter spp. (4.2%), Enterobacter spp. (11%), Pseudomonas spp. (20%), Proteus spp. (13%), *Morganella morganii*(1.7%) and Providencia (0.04%).

In our study, antimicrobial susceptibility was done by disk diffusion method following CLSI 2016 recommendation, (Imipenem 10µg: S: ≥23, I: 20-22, R: ≤19), (Meropenem: 10µg: S: ≥23, I: 20-22, R: ≤19), (Ertapenem: 10µg: S: ≥ 22, I: 19-21, R: ≤ 18) for Enterobactericae , For *Pseudomonas aeruginosa* (imipenem 10µg: S: ≥19mm, I: 16-18mm, R: ≤15mm), (Meropenem 10µg: S: ≥19mm, I: 16-18mm, R: ≤15mm), For Acinetobacter species (imipenem 10µg: S: ≥22mm, I: 19-21mm, R:≤18mm), (Meropenem 10µg: S: ≥18mm, I: 15-17mm, R: ≤14mm).

Carbapenem susceptibility pattern showed that 49(81.6%) were Imipenem resistant, 46(76.7%) were Meropenem resistant and 50 (83.3%)were ertapenem resistant. ***Campanaet al.*** [12] reported that a total of 131 Enterobacteriaceae strains were tested. Of those, 62 (47.33 %) strains were resistant to at least one antibiotic. Twenty-five (25/62) (40%) strains were resistant to meropenem, followed by ertapenem 28/62 (45%) and resistant to both (24/62) (38.7%).

Sahin et al. [9] reported that of the 43 strains, all were ertapenem-resistant, 95.3 % were meropenem-resistant and 83.7 % were imipenem-resistant. A resistance rate of 97.7 % to ertapenem was detected using antimicrobial gradient test technique, whereas the resistance rate was 100 % accordingto the VITEK®2 automated system. Additionally, meropenem resistance was calculated using the antimicrobial gradient test (93 %) and the automated system (90.7 %).

In our stud, Genetic distribution for carbapenemase genes among the study group was as follow Out of *Klebseilla pneumonia* 4(13.0%) isolates were negative, 2(5.7%) isolate was positive for VIM, 15(43%) were positive for KPC, VIM, 8(22.9%) isolates were positive for KPC, VIM, OXA ,2(5.7%) isolate was VIM, NDM, 2(8.7%) isolates were positive for KPC, GES.

Out of Pseudomonas 4(50.0%) isolates were positive for KPC, VIM, 2(25%) isolates were positive for VIM, NDM, 2(25%) isolates were positive for VIM, IMP.

Out of Enterobacter spp., 7(58.3%) isolates were positive for KPC, VIM, 4(33.3%`) were negative for PCR. Out of Acinetobacter spp., 1(100%) was positive for VIM, IMP. Out of *Proteus mirabilis* 2(66.6%) isolate was positive for KPC, VIM, 1(33.3%) was positive for VIM. The only one Serratia (100%) isolate was positive for KPC, VIM, OXA. ***Hombach et al.*** [13] reported that in total, 51 carbapenemase genes were detected in 252 Enterobacteriaceae isolates (20.2%), i.e., 13 bla_{KPC}, 1 bla_{IMI}, 1 bla_{IMP}, 6 bla_{VIM}, 10 bla_{NDM}, 1 bla_{GIM}, and 19 bla_{OXA-48}-like.

In another study, ***Dortet et al.*** [14] found that from collection of 150 enterobacterial isolates, including 132 isolates with decreased susceptibility to at least one carbapenem molecule, and 55 non carbapenemase producers: 21 KPC producers, 21 NDM producers, 17 VIM producers, 11 IMP producers, 16 OXA-48 producers and 9 OXA-48-like producers (OXA-162, OXA-181, OXA-204, OXA-232 and OXA-244).

Ready-to-use rapidec carba NP gave high sensitivity and specificity for reliable detection of carbapenemases in Enterobacteriaceae, *Acinetobacter baumannii* and P. *aeruginosa* isolates[15].

Without specialized equipment, it detects the pH change caused by the breakdown of imipenem in a solution containing lysed test bacteria (thus by passing confounding factors caused by membrane changes) [16].

In our study, rapidec carba NP gave 100% sensitivity, specificity, positive predictive value and negative predictive value. All strains had been characterized for their β-lactamase content at the molecular level. This was in agreement

with ***Nordmann et al.*** [1] who reported that the test's specificity and sensitivity were 100% when results were compared with those from molecular-based methods.

Hombach et al. [13] reported that overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the rapidec Carba NP test after 120 min of incubation were 90.2%, 100%, 100%, and 97.6%, respectively, when the manufacturer's instructions were followed. Four of 5 false-negative results occurred with OXA-48-like enzymes, in one *Citrobacter koseri* isolate, two E. coli isolates, and one *K. pneumoniae* isolate; one false-negative result was obtained with a VIM-type enzyme.

In a comparison between RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test for biochemical detection of carbapenemase-producing Enterobacteriaceae ***Dortet et al.***[14], reported that the RAPIDEC (®) CARBA NP detected all carbapenemase producers except a single OXA-244 producer. Using the Rapid CARB Screen (®), one KPC-2, two NDM-1, one OXA-48 and five OXA-48 variant producers gave equivocal results and one OXA-244 producer was not detected.

Using the Carba NP test, the same OXA-244 producer was not detected and one OXA-181 producer and one OXA-244 producer gave equivocal results. Sensitivity and specificity were 99% (95% CI 94.3%-99.8%) and 100% (95% CI 93.5%-100%), respectively, for the RAPIDEC (®) CARBA NP test, 89.5% (95% CI 81.7%-94.2%) and 70.9% (95% CI 57.9%-81.2%) for the Rapid CARB Screen (®) and 96.8% (95% CI 91.1%-98.9%) and 100% (95% CI 93.5%-100%) for the Carba NP test. The impact of the use of an adequate bacterial inoculum for obtaining the optimal performance with the RAPIDEC (®) CARBA NP was noted.

In our study, delayed positive results in 4 isolates containing OXA-48 were noticed. Reading the test after 30 minutes gave false negative or weak positive results while after 120 min of incubation yielded positive results and this is in accordance with manufacturer's instructions.

Hombach et al. [13] reported that after an incubation time of 30 min, the sensitivity was 49%, i.e. (27/51) carbapenemase producers tested negative. Nineteen (70%) of 27 isolates with negative readings after 30 min of incubation harbored OXA-48-like enzymes, four (15%) harbored VIM enzymes, three (11%) harbored NDM enzymes, and one harbored a KPC enzyme. Twenty-two of the 27 isolates with negative readings after 30 min of incubation yielded positive test results after 120 min of incubation. No fading of the color reaction was seen after 120 min for tests that were already positive after 30 min of incubation.

Srisrattakarn et al. [17]reported that the strip test gave false negative or weak positive results in OXA-48 and OXA-181 producing Enterobacteriaceae and IMP-14a-producing *P*. aeuroginosa isolates. These results were similar to reports of ***Tijet et al.***[18] and they suggested that low hydrolytic activity of these enzymes was the cause [19].

Maurer et al. [20] also reported sensitivity of 78.9% for CPE detection by the Carba NP test. It was suggested that the sensitivity of the Carba NP test may depend on the prevalence of carbapenemases with low hydrolysis activity to imipenem in each area (particularly OXA carbapenemases or some IMP enzymes), low carbapenemase gene expression in some isolates, mucoid colonies (difficulties in protein extraction), species-specific traits (due to the unknown impact of different genetic backgrounds), types of media agar or imipenem powder.

Dortet et al. [14]stated that some molecular methods such as PCR cannot distinguish true OXA-48-like carbapenemases (e.g.OXA-48, OXA-181) from OXA-48-like non-carbapenemase variants (e.g. OXA-163, OXA-405) unless nucleotide sequencing is performed. Therefore, Carba NP negative results in blaOXA-48-like-carrying isolates may be true negatives due to the presence of non carbapenemase OXA-48-like enzyme.

In our study, the cost was (7.5 USD) per test that is considered relatively inexpensive while ***Garg et al., 2015*** reported that they cost 1 and 5 USD per test for the original carba NP test and the paper strip test respectively.

In summary, the Rapidec Carba NP test was demonstrated to be a useful tool for the reliable confirmation of carbapenemase-producing Enterobacteriaceae isolates. The test should be read strictly after 120 min of incubation, particularly in cases in which $bla_{\text{OXA-48}}$ is suspected

References:-

1. Nordmann P, Poirel L, Dortet L (2012): Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. Sep;18(9):1503-7.
2. Nordmann P and Poirel L (2013): Strategies for identification of carbapenemase-producing Enterobacteriaceae**.** J AntimicrobChemother.; 68(3):487-9.
3. Edwards SJ, Emmas CE, Campbell HE (2005): Systematic review comparing meropenem with imipenem plus cilastatin in the treatment of severe infections. Curr Med Res Opin. 21 (5): 785–94.
4. Ederer GM and Clark M (1970): Motility-indole-ornithine medium**.**ApplMicrobiol.; 20(5):849-50**.**
5. Christensen H, Korczak BM, Bojesen AM, Kuhnert P, Frederiksen W, Bisgaard M (2011): Classification of organisms previously reported as the SP and Stewart-Letscher groups, with descriptions of Necropsobacter gen. nov.and of Necropsobacter rosorum sp. nov.for organisms of the SP group. Int J SystEvolMicrobiol.; 61(Pt 8):1829-36.
6. Pao S, Davis CL, Friedrich LM, Parish ME (2002): Utilization of fluorogenic assay for rapid detection of Escherichia coli in acidic fruit juice. J Food Prot.;65(12):1943-8.
7. Osterblad M, Hakanen AJ, Jalava J (2014): Evaluation of the Carba NP test for carbapenemase detection**.**Antimicrob Agents Chemother.; 58(12):7553-6.
8. Maurer FP, Castelberg C, Quiblier C, Bloemberg GV, Hombach M (2015): Evaluation of carbapenemase screening and confirmation tests with Enterobacteriaceae and development of a practical diagnostic algorithm**.** J ClinMicrobiol. Jan;53(1):95-104.
9. Sahin K, Tekin A, Ozdas S, Akin D, Yapislar H, Dilek AR, Sonmez E (2015): Evaluation of carbapenem resistance using phenotypic and genotypic techniques in Enterobacteriaceae isolates**.** Ann ClinMicrobiolAntimicrob.; 14: 44.
10. Tan TY, Hsu LY, Koh TH, Ng LS, Tee NW, Krishnan P, Lin RT, Jureen R (2008): Antibiotic resistance in gram-negative bacilli: a Singapore perspective**.** Ann Acad Med Singapore; 37(10):819-25**.**
11. Parveen M, Ghalib RM, Khanam Z, Mehdi SH, Ali M (2010): A novel antimicrobial agent from the leaves of Peltophorumvogelianum (Benth.). Nat Prod Res.; 24(13):1268-73.
12. Campana EH, Xavier DE, Petrolini FV, Cordeiro-Moura JR, Araujo MR, Gales AC (2016): Carbapenem-resistant and cephalosporin-susceptible: a worrisome phenotype among Pseudomonas aeruginosa clinical isolates in Brazil. Braz J Infect Dis. Dec 1. pii: S1413-8670(16)30560-8. doi: 10.1016/j.bjid.2016.10.008. [Epub ahead of print]
13. Hombach M, von Gunten B, Castelberg C, Bloemberg GV (2015): Evaluation of the Rapidec Carba NP Test for Detection of Carbapenemases in Enterobacteriaceae. J Clin Microbiol.;53(12):3828-33.
14. Dortet L, Agathine A, Naas T, Cuzon G, Poirel L, Nordmann P (2015): Evaluation of the RAPIDEC® CARBA NP, the Rapid CARB Screen® and the Carba NP test for biochemical detection of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother.;70(11): 3014-22.
15. Poirel L and Nordmann P (2015): Rapidec Carba NP Test for Rapid Detection of Carbapenemase Producers. J Clin Microbiol.;53(9):3003-8.
16. Österblada M, Hakanena AJ, Jalavaa J (2014): Evaluation of the Carba NP Test for Carbapenemase Detection. Antimicrobial Agents and Chemotherapy; 58(12): 7553–7556.
17. Srisrattakarn A, Lulitanond A, Wilailuckana C, Charoensri N, Wonglakorn L, Piyapatthanakul S, Supajeen A, Chanawong A (2016): Modification and evaluation of the Carba NP test by use of paper strip for simple and rapid detection of carbapenemase-producing Enterobacteriaceae**.** World J MicrobiolBiotechnol.; 32(7):117.
18. Tijet N1, Boyd D, Patel SN, Mulvey MR, Melano RG (2013): Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa**.**Antimicrob Agents Chemother.; 57(9):4578-80.
19. Rimrang B, Chanawong A, Lulitanond A, Wilailuckana C, Charoensri N, Sribenjalux P, Phumsrikaew W, Wonglakorn L, Kerdsin A, Chetchotisakd P (2012): Emergence of NDM-1- and IMP-14a-producing Enterobacteriaceae in Thailand**.** The Journal of antimicrobial chemotherapy. PMID: 22796889.
20. Maurer FP, Castelberg C, Quiblier C, Bloemberg GV, Hombach M (2015): Evaluation of carbapenemase screening and confirmation tests with Enterobacteriaceae and development of a practical diagnostic algorithm. J ClinMicrobiol.; 53(1):95-104.
21. Garg A, Garg J, Upadhyay GC, Agarwal A, Bhattacharjee A (2015): Evaluation of the Rapidec Carba NP Test Kit for Detection of Carbapenemase-Producing Gram-Negative Bacteria. Antimicrob Agents Chemother.; 59(12):7870-2.