Diagnostic Efficacy of the Carba NP Strip Test for Carbapenemase Detection

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Background and study aim: Carbapenemase-producing gram-negative bacteria are widely distributed in hospitals and cause nosocomial infections with high mortality rates. The current study aimed to assess the diagnostic performance of the Carba NP strip test versus tube method, and to determine the frequency of carbapenemase genes.

Materials and Methods: In this prospective study, we performed the Carba NP strip test and Carba NP tube method in hundred gram-negative isolates (60 *Klebsiella pneumoniae*, 26 *Pseudomonas*, and 14 *E.coli*). We detected bla_{KPC}, bla_{VIM}, bla_{OXA-48}, bla_{GES}, and bla_{IMP} genes by the Real-time PCR.

Results: Out of the 80 meropenem-resistant isolates, bla_{VIM} (66/80, 82.5%), followed by the bla_{OXA-48} gene (60/80, 75%), were the most prevalent gene, whether as a single gene or coexpressed with other genes. One hundred percent of the *Klebsiella pneumoniae* and *E. coli* isolates harboured the bla_{OXA-48} gene. *Pseudomonas* isolates were positive for the bla_{VIM} gene as a single gene. The Carba NP strip method exhibited high sensitivity and specificity, but the tube method had a higher diagnostic performance in *Pseudomonas* isolates.

Conclusion: The current study highlights that Carba NP strip test can be a reliable and excellent alternative to other tedious and expensive standard techniques, making carbapenem resistance diagnosis reachable to routine laboratories. The Carba NP strip test is simple, inexpensive, and rapid. It can be implemented in low-resource healthcare settings for early detection of carbapenem resistance, hence initiating proper therapy. Further studies on broad scales are recommended to study the effect of other carbapenemase genes on sensitivity and specificity.

INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) isolates mediated by carbapenemase production are broadly disseminated **[1]**. Carbapenemase producers frequently manifest multidrug resistance. They can hydrolyze a broad spectrum of β-lactams, making infection treatment difficult. They can propagate quickly in hospitals, causing nosocomial infections with elevated death rates **[2]**.

Different methods are available for the rapid diagnosis of carbapenemase production, such as the carbapenemase inactivation method, chromogenic media, MALDI-TOF technology, and molecular techniques **[3]**. Detection of carbapenemase genes via molecular methods is pricey and demands particular equipment. Moreover, phenotypic carbapenemase confirmatory tests may take up to 24 hours. Therefore, there is a necessity to find a quick and inexpensive phenotypic test for the diagnosis of CRE, allowing the initiation of proper antimicrobial, and implementation of infection control measures to restrain their pervasion **[4-6]**.

A quick chromogenic test was developed for detecting carbapenemases called Carba NP. This test depends on carbapenemases' ability to break the beta-lactam ring of imipenem, yielding an acid which can be identified by the shift of phenol red color from red to yellow or orange. For detecting carbapenemases in *Enterobacteriaceae*, it exhibited 100% sensitivity and specificity. In

Pseudomonas, the results were 94.4 and 100%, respectively **[7]**.

Also, an acidimetric paper strip test was tested for detecting beta-lactamase in *Neisseria gonorrhoeae* and *Haemophilus influenzae*. It proved to be easy and speedy **[8]**. It is desirable to use paper as a medium for colorimetric tests because it is cheap and environmentally friendly, as it can be incinerated **[9]**. Thus, similarly, the Carba NP test modification using filter paper strips can be established **[10]**.

The present work aimed to assess the diagnostic performance of the Carba NP strip test versus the CLSI tube method **[4]**, compared with PCR method, to confirm carbapenemase activity, and to determine the frequency of carbapenemase genes bla_{KPC}, bla_{VIM}, bla_{OXA-48}, bla_{GES}, and bla_{IMP}.

MATERIAL AND METHODS

Study design:

This prospective study was carried out to assess the performance of the Carpa NP strip test for detecting carbapenem-resistant gram-negative bacteria compared to PCR method during the period from June 2020 to December 2020.

Bacterial strains:

A total of 100 gram-negative isolates, randomly selected from various clinical specimens and submitted routinely for culture and susceptibility to the Main Microbiology Laboratory of Ain Shams University Hospitals (ASUH), were included. Eighty isolates were carbapenem-resistant, and twenty carbapenem-susceptible isolates were used as a control group. All isolates were identified by Vitek2C (Biomerieux, France). The minimum inhibitory concentration (MIC) of meropenem was measured by the broth microdilution method (BMD) according to CLSI **[4]**.

Carba NP test:

The Carba NP test was done in accordance with CLSI **[4]**. Two tubes were designated (a and b) for each studied isolate, quality control organism, and uninoculated reagent control. We added 100 µl of bacterial protein extraction reagent (BPE) to each tube (Thermo Scientific, USA). Then, 1 µl of loopful bacteria from an overnight culture, on blood agar plates, was dissolved in both tubes by vortexing for 5 seconds. We added 100 µl of each solution A and solution B to tubes (a and b), and tube b, respectively. Uninoculated reagent control tubes contained only BPE reagent. Tubes were vortexed well and incubated at 35+2°C for up to 2 hours. The results were recorded after 1 minute, 5 minutes, 1 hour, and 2 hours.

Interpretation:

Both uninoculated control tubes (a and b) and the inoculated tube (a) were red to red-orange. If any color else was observed, the test was regarded as invalid. Red or red to orange inoculated tube (b) was negative for carbapenemase production (figure 1). Light orange, dark yellow or yellow inoculated tube (b) was regarded as positive (figure 2) **[4]**.

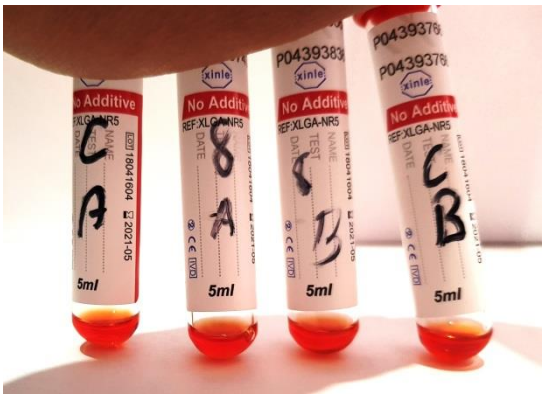

Figure 1: Uninoculated Carba NP test tubes used as control tubes labelled C (A and B) and inoculated Carba NP test tubes labelled 8 (A and B) giving negative results.

Figure 2: Uninoculated Carba NP test tubes used as control tubes labelled C (A and B) and inoculated Carba NP test tubes labelled 11 (A and B) giving positive results (yellow color).

Carba NP strip test:

The Carba NP strip test was carried out utilizing solutions A and B, similar to the CLSI Carba NP test. Modulations to the technique using filter papers were performed according to Ho *et al.* **[10]**. Filter papers were cut into squares 15 mm

in size and fixed using adhesive tape onto a petri dish to help with bacterial application. Each strip was moistified with 50 µl of solution A (control strip) or solution B (test strip). Thereafter, one µl loopful from an overnight culture of each tested organism was rubbed in a circle of 5-7 mm diameter on the control and test strips. Rapid strip dryness was avoided by covering the plates with lids, and then they were incubated at 37°C. The results were recorded after one minute and five minutes.

Interpretation:

Any color shift (yellow or light yellow) on the test strip was reported as positive. In case the color of the test strips remained red at 5 minutes, the result was negative. If the control strip became yellow, the test was invalid (figures 3, 4).

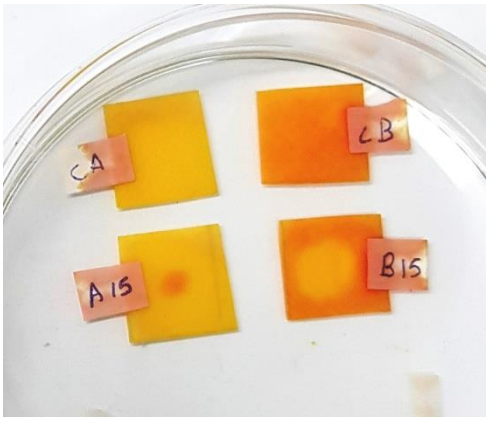

Figure 3: Uninoculated control strip (CA) with solution A. Uninoculated test strip (CB) with solution B. Inoculated control strip (A 15) with slight pink color from the mucoid *Klebsiella* colony. Inoculated test strip (B 15) giving a positive result with a color change from red to yellow.

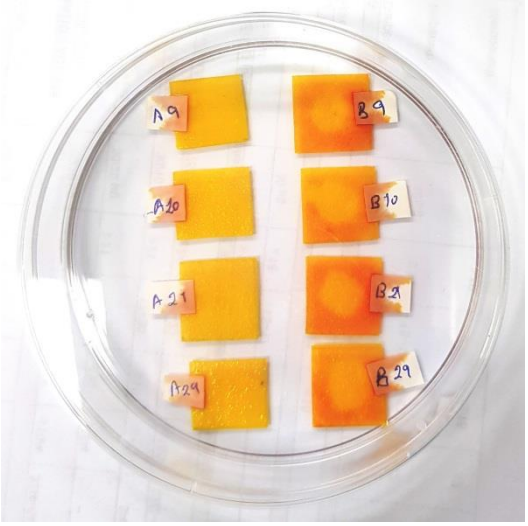

Figure (4): Inoculated Carba Np test strips giving negative results (no color change in test strip).

Detection of carbapenemase genes with multiplex PCR:

All 100 isolates were subjected to multiplex PCR to detect the *bla*$_{GES}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$, and *bla*$_{KPC}$ genes. DNA extraction, using a QIAamp DNA Mini Kit (Qiagen, USA), was performed as recommended by the manufacturer. The thermal cycling conditions and a melt program were performed according to Shabban et al. **[11]**. Amplification products were detected using QuantiTect SYBR Green PCR Kits (Qiagen, USA) added to the master mix.

In the current study, the forward and reverse primers for detecting *bla*$_{KPC,}$ *bla*$_{GES}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$, and *bla*$_{VIM}$ genes were designed as per Monteiro and colleagues **[12]**. They were supplied by Qiagen (USA).

K. pneumoniae ATCC1705 was used as a positive control, while *K. pneumoniae* ATCC1706 was a negative control. All the quality control reference strains were supplied by (Remel, UK).

Statistical Analysis:

Data entry and statistical analysis of the collected data were performed by SPSS version 23. Descriptive and analytical procedures were evaluated using Fishers exact test to examine the relationship between two qualitative variables when the expected count was less than 5 in more than 20% of cells. A statistically significant difference was considered at a p value ≤ 0.05. The diagnostic performance of the Carba NP test was determined using diagnostic sensitivity, diagnostic specificity, predictive value for a positive test (PPV), and predictive value for a negative test (NPV).

RESULTS

Spectrum of bacterial isolates:

In the current study, we used 100 gram-negative isolates. They included sixty *Klebsiella pneumoniae* isolates (60/100, 60%), twenty-six *Pseudomonas aeruginosa* isolates (26/100, 26%), and fourteen *E. coli* isolates (14/100, 14%).

Meropenem MIC by BMD:

Eighty isolates were resistant to meropenem (80%). Most of the resistant isolates were *Klebsiella pneumoniae* (52/80, 65%), followed

by *Pseudomonas aeruginosa* (20/80, 25.0%) and *E. coli* (8/80, 10%).

Genetic types of isolates:

Regarding the results of PCR, Table (1) summarizes the distribution of different genes among the various types of isolates in the current study. Among the eighty meropenem-resistant isolates, twenty-four were positive for a single gene; four *Klebsiella pneumoniae* isolates were positive for bla_{OXA-48}, and 20 *Pseudomonas* isolates were positive for bla_{VIM}. In contrast, fifty-six isolates (eight *E.coli* and 48 *Klebsiella pneumoniae*) were positive for at least two genes. The bla_{IMP} gene was not found in any of our isolates. All twenty meropenem-susceptible isolates were negative by PCR.

Carba NP tube and Carba NP strip test:

Regarding Carba NP tube test results, 68 isolates were positive (68/100, 68%); 44/68 (64.7%) *Klebsiella pneumoniae* isolates, 18/68 (26.5%) *Pseudomonas aeruginosa* isolates, and 6/68 (8.8%) *E. coli* isolates.

For the Carba NP strip test results, 74 isolates were positive (74/100, 74%); 50 (67.6%) *Klebsiella pneumoniae* isolates, 16 (21.6%) *Pseudomonas aeruginosa* isolates, and 8 (10.8%) *E. coli* isolates.

A highly statistically significant correlation was found between PCR and the Carba NP tube method and strip test (Table 2). Table (3) illustrates the correlation between PCR and the Carba NP tube method and strip test among the different organism species in the current study.

Table (4) summarizes the diagnostic performance of the Carba NP tube method and the Carba NP strip test. Although both tests showed an overall 100% specificity, the Carba NP strip test exhibited a higher sensitivity (92.5%) than the Carba NP tube test (85%).

Table (5) shows the sensitivity of the Carba NP tube and Carba NP strip methods to detect different carbapenemase genes in the present study. Carba NP strip method exhibited an overall higher sensitivity than the tube method, except for bla_{GES}, for which the tube method displayed 100% sensitivity.

For the Carba NP tube test, the reading time of positive results for the tested isolates was recorded after 1 minute, 5 minutes, 1 hour, and 2 hours. Out of the 68 isolates expressing positive results, two isolates were positive at 1 minute (2/68, 2.9%), eight isolates were positive at 5 minutes (8/68, 11.8%), fourteen isolates were positive at 1 hour (14/68, 20.6%), and 44 isolates were positive at 2 hours (44/68, 64.7%).

For the Carba NP strip test, the results of the tested isolates were recorded at 1 minute and 5 minutes. Out of the 74 positive isolates, fourteen were positive at 1 minute (14/74, 18.9%), and sixty were positive at 5 minutes (60/74, 81.1%).

The data in table (6) show a statistically significant difference between both Carba NP tube and strip tests regarding the reading time of positive results, with the strip test being more rapid, as 100% of cases were read under 5 minutes.

Table (1): Carbapenemase genes of studied isolates.

Carbapenemase genes	Species		
	Klebsiella pneumoniae	***E.coli***	***Pseudomonas aeruginosa***
bla_{OXA-48}	4		
bla_{VIM}			20
bla_{OXA-48} + bla_{VIM}	10	8	
bla_{KPC}+ bla_{OXA-48}	4		
bla_{OXA-48} + bla_{VIM}+ bla_{KPC}	12		
bla_{GES}+ bla_{OXA-48} + bla_{VIM}	6		
bla_{GES}+ bla_{OXA-48} + bla_{KPC}	6		
bla_{GES}+ bla_{OXA-48} + bla_{VIM}+ bla_{KPC}	10		

Table (2): Correlation between PCR and both Carba NP tube and strip method.

Test	Result	PCR				P-value	Sig.
		Negative (n=20)		Positive (n=80)			
		No.	%	No.	%		
Carba NP tube	Negative	20	100.0%	12	15.0%	0.000	HS
	Positive	0	0.0%	68	85.0%		
Carba NP Strip test	Negative	20	100.0%	6	7.5%	0.000	HS
	Positive	0	0.0%	74	92.5%		

Table (3): Carba NP tube and strip method among the different organism species in relation to PCR.

Klebsiella (n=60)		Carbapenemase genes by PCR				P-value	Sig.
		Negative (n=8)		Positive (n=52)			
		No.	%	No.	%		
Carba NP tube	Negative	8	100.0%	8	15.4%	0.000	HS
	Positive	0	0.0%	44	84.6%		
Strip test	Negative	8	100.0%	2	3.8%	0.000	HS
	Positive	0	0.0%	50	96.2%		
Pseudomonas **(n=26)**		**Carbapenemase genes by PCR**				**P-value**	**Sig.**
		Negative (n=6)		**Positive (n=20)**			
		No.	**%**	**No.**	**%**		
Carba NP tube	Negative	6	100.0%	2	10.0%	0.000	HS
	Positive	0	0.0%	18	90.0%		
Strip test	Negative	6	100.0%	4	20.0%	0.000	HS
	Positive	0	0.0%	16	80.0%		
E-Coli **(n=12)**		**Carbapenemase genes by PCR**				**P-value**	**Sig.**
		Negative (n=6)		**Positive (n=6)**			
		No.	**%**	**No.**	**%**		
Carba NP tube	Negative	6	100.0%	2	25.0%	0.009	HS
	Positive	0	0.0%	6	75.0%		
Strip test	Negative	6	100.0%	0	0.0%	0.000	HS
	Positive	0	0.0%	8	100.0%		

Table (4): Diagnostic performance of Carba NP tube test and Carba NP strip test compared to PCR.

Carba NP tube	TP	TN	FP	FN	Accuracy	Sensitivity	Specificity	PPV	NPV
All cases	68	20	0	12	90.0	85.0	100.0	100.0	66.7
Klebsiella	46	8	0	6	90.0	84.6	100.0	100.0	57.1
Pseudomonas	18	6	0	2	92.3	90.0	100.0	100.0	75.0
E.coli	6	6	0	2	100.0	75.0	100.0	100.0	75.0
Strip test	**TP**	**TN**	**FP**	**FN**	**Accuracy**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
All cases	74	20	0	6	94.0	92.5	100.0	100.0	76.9
Klebsiella	50	8	0	2	96.7	96.2	100.0	100.0	80.0
Pseudomonas	16	6	0	4	84.6	80.0	100.0	100.0	60.0
E.coli	8	6	0	0	100.0	100.0	100.0	100.0	100.0

TP: True positive, **TN:** True negative, **FP:** False positive, **FN:** False negative, **PPV:** Predictive value for a positive test, **NPV:** Predictive value for a negative test.

Table (5): The sensitivity of Carba NP tube and Carba NP strip methods to detect different types of carbapenemase genes.

Gene	Carba NP Strip test				Sensitivity (%)	95% CI
	Negative(n=6)		Positive(n=74)			
	No.	%	No.	%		
***bla*OXA-48**	2	33.3%	58	78.4%	96.67	(0.8847-0.9959)
***bla*KPC**	0	0.0%	32	43.2%	100	(0.8911- 1)
***bla*VIM**	6	100.0%	60	78.4%	90.91	(0.8126-0.9659)
***bla*GES**	2	33.3%	20	27%	90.91	(0.7084-0.9888)
Gene	**Carba NP tube**				**Sensitivity (%)**	**95% CI**
	Negative (n=12)		**Positive (n=68)**			
	No.	**%**	**No.**	**%**		
***bla*OXA-48**	10	83.3%	50	73.5%	83.33	(0.7148-0.9171)
***bla*KPC**	4	33.4%	28	41.2%	87.5	(0.7101-0.9649)
***bla*VIM**	10	83.3%	56	79.4%	84.85	(0.7390-0.9249)
***bla*GES**	0	0.0%	22	32.3%	100	(0.8456- 1)

Table (6): Correlation between Carba NP tube test and strip method regarding time of reading positive results.

Results		Test				P-value	Sig.
		Carba Np tube		Strip test			
		No. = 68	%	No. = 74	%		
Time(min)	Under 5 min	10	14.7%	74	100%	0.000	HS
	Over 5 min	58	85.3%	0	0.0%		

DISCUSSION

The carbapenem resistance in gram-negative rods is of great concern because of the limited options available to treat the infections caused by these organisms **[12]**. Carbapenemases in gram-negative organisms such as *Enterobacteriaceae* and *Pseudomonas* confer resistance to a wide diversity of β-lactams. These genes can disseminate among bacteria by self-conjugative plasmids. These plasmids bear other resistance determinants, and this results in the resistance to numerous antibiotic classes, such as fluoroquinolones, aminoglycosides and cotrimoxazole **[13]**.

Speedy recognition of carbapenemase-producing organisms is mandatory for prompt diagnosis, therapy, and application of infection control measures to prohibit their propagation **[13]**.

The present work aimed to assess the diagnostic performance of the Carba NP strip test versus the CLSI tube method, compared with PCR method, to confirm carbapenemase activity, and to determine the frequency of carbapenemase genes *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, *bla*$_{GES}$, and *bla*$_{IMP}$.

Regarding the Carba NP tube test, the results were read at 1 minute, 5 minutes, 1 hour, and 2 hours. The readings were 2.9%, 11.8%, 20.6%, and 64.7%, respectively. The test had overall sensitivity, specificity, PPV, and NPV of 85%, 100%, 100%, and 66.7%, respectively.

Our results were in concordance with those of other researchers. They reported similar sensitivity and specificity to ours **[14-16]**.

On the other hand, Tijet and colleagues reported a sensitivity and NPV of 72.5% and 69.2%, respectively **[17]**. The difference between our reported sensitivity and specificity and other researchers may be attributed to the usage of different inoculation media, as they used Muller Hinton agar, whereas in our study, blood agar was used as recommended by CLSI guidelines.

The variance in the Carba NP test sensitivity might also be related to numerous factors, such as the dissimilarity in the frequency rate of carbapenemases, with decreased hydrolyzing activity of some types to imipenem, and decreased gene expression in some bacteria. Additionally, mucoid colonies, causing hardness in protein extraction, may be another possible explanation for such variation **[17-19]**. Hence, It

is not easy to infer the false negativity of the Carba NP test, and more investigations are needed.

In the current study, Carba NP strip test results were read at 1 minute and 5 minutes. The overall sensitivity, specificity, PPV, and NPV were 92.5%, 100%, 100% and 76.9%, respectively. The Carba NP tube test generally was bestead than the Carba NP tube method. However, the Carba NP tube method was superior to the strip method for diagnosing *Pseudomonas* isolates. Our findings were comparable to other researchers **[10, 20-21]**.

The primary significance of the present work was the disparity in diagnostic timing between the strip and tube methods, which was less than 5 minutes for 100% of isolates for the strip test, and 85.29% of isolates were more than 5 minutes, reaching up to two hours for the tube method. Similarly, the evaluation of timing conducted by Ho and colleagues yielded the same results, with the strip test being more rapid, with 100% of isolates under 5 minutes and more than 70% of isolates over 5 minutes reaching up to 2 hours with the Carba NP tube method **[10]**.

Based on our findings, It is easy to conduct the strip test as it is an effortless and cheap method. Moreover, little amount of reagent is needed per test and no need for BPE. In our study, the strip test costs around 0.5 USD per test, making it a very inexpensive test for carbapenemase detection in a routine laboratory.

Among the observed limitations of the strip test in the current study was reading the color change on the strip. We overcame this by using a white background when reading the results. In the research conducted by Ho and coworkers, they also reported that strip reactions resulted in an interpretive discrepancy in color readings between the two blinded observers, and using a color chart helps interpret strip results **[10]**.

In the current work, using real-time multiplex PCR, carbapenemase genes were detected in the 80 isolates resistant to meropenem. None of the susceptible isolates harboured the carbapenemase genes. bla_{VIM} (66/80, 82.5%), followed by the bla_{OXA-48} gene (60/80, 75%), whether as a single gene or coexpressed with other genes, was the most prevalent gene among our studied isolates. The bla_{IMP} gene was not found in any of our tested isolates. For *Enterobacteriaceae*, 100% of the *Klebsiella* and *E. coli* isolates were positive for the bla_{OXA-48} gene. It was coexpressed with other genes in the majority of the isolates. It is worth noting that *Pseudomonas* isolates were positive for the bla_{VIM} gene as a single gene, and no coexpression was observed.

These results agreed with several studies, as the bla_{OXA-48} gene was the predominant carbapenemase among their *Enterobacteriaceae* isolates **[22-24]**. Additionally, Taher from Egypt did not detect the bla_{IMP} gene in any tested isolates **[24]**. In line with our results, other researchers from Lebanon, Egypt, and Germany showed that bla_{VIM} was the only isolated gene among their tested *Pseudomonas* isolates **[25-27]**.

On the other hand, other investigators observed different gene distributions. Okoche *et al*. in Uganda found that among 67 carbapenem-resistant isolates, the most prevailing genes was bla_{VIM} (10.7%), bla_{OXA-48} (9.7%), bla_{IMP} (6.1%) and bla_{KPC} (5.1%) **[28]**. Additionally, Yang and coworkers in China observed that the most common carbapenemase gene identified among *Enterobacteriaceae* isolates was bla_{KPC}, followed by bla_{GES} **[29]**. Zhou *et al*. in China also reported that bla_{KPC} was the most frequent carbapenemase gene among *Enterobacteriaceae* **[30]**. Lucena and colleagues in Germany found that bla_{VIM} was the most frequent carbapenemase gene in *Enterobacteriaceae* **[31]**. These findings may be due to differences in the prevalence rate of carbapenemase genes in different geographical areas.

Multidrug resistance in Egypt, especially carbapenem resistance, is distressing, and it can be attributed to previous antibiotic intake (especially carbapenems), a long hospital admission period, empirical treatment by carbapenem antibiotics, and the unrestricted over-the-counter sale of all types of antibiotics **[32]**. This can explain the multiple resistance gene coexpression detected in our study. Other studies also reported the coexpression of numerous genes in their isolates **[24, 32-34]**.

In the current study, the Carba NP strip showed a higher sensitivity than the tube method to detect the different tested carbapenemase genes. The sensitivity of the Carba NP strip varied from 90.91% to 100%. However, the tube method was superior to the strip method in detecting the bla_{GES} gene.

Although in our study, the strip method failed to detect two isolates with *bla*$_{OXA-48}$, other studies contradicted this finding. Srisrattakarn and colleagues from Thailand reported that the strip method, in *Enterobacteriaceae* gave negative results in 5 of the 6 isolates OXA-48 and OXA-181 **[20]**. Additionally, Ho *et al*. from Hong Kong mentioned that using the strip test, they failed to detect more than two-thirds of the OXA-48-producing isolates **[10]**.

It should be taken into consideration that some observations and limitations were noticed during our work: 1- reading of test results needs experience as the colour contrast is not strong and needs a white background for clear differentiation between colours; 2- the test requires fast inoculation of tested isolates after applying solutions A and B on the strips to avoid dryness of test strips, which will interfere with test reading; 3- the material used to tape the strips to the plates should not heavily absorb the solutions after being applied on strips to avoid interference with test reading; 4- due to the different constitution between solution A and solution B, applying solution A on filter paper gave a lighter colour. Increasing phenol red concentration did not change the color, so we followed the initial recommended concentration; 5- mucoid isolates, especially *Klebsiella*, gave a slight red to pink color on solution A strip, and it was not considered invalid as invalidity is the change from red to yellow on solution A strip. Additionally, our study did not include all the other carbapenemase genes.

CONCLUSION

The Carba NP strip test is a simple and affordable method with high sensitivity and specificity. Although its sensitivity is higher than that of the tube method, the tube method took the upper hand in regard to the *Pseudomonas* isolates.

The modified strip method is a rapid test providing results within 5 minutes instead of 2 hours for the tube method, allowing the rapid initiation of proper treatment. It is also easier to eliminate because the materials required for testing are simple and minimal. Moreover, this test does not need trained personnel or special skills for performing the test and reading the results.

Therefore, we recommend the Carba NP strip test for early diagnosis of carbapenemase genes, especially in low-resource health care settings. Further studies on broad scales with large sample sizes are recommended to study the effect of other carbapenemase genes on sensitivity and specificity.

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Conflict of interest: None

Ethics approval and consent to participate: This research was approved by the Ethical Research Committee, Faculty of Medicine, Ain Shams University. (ethical approval number: FWA 000017585), 2019.

HIGHLIGHTS

- This study evaluated the performance of the Carba NP strip test versus the tube method and determined the frequency of carbapenemase genes among Carbapenemase-producing gram-negative isolates.
- Both methods showed an overall 100% specificity, but the Carba NP strip test exhibited a higher sensitivity (92.5%) than the tube method (85%). The tube method had a higher diagnostic performance in Pseudomonas isolates. *bla*$_{VIM}$ (66/80, 82.5%), followed by the *bla*$_{OXA-48}$ gene (60/80, 75%), were the most prevalent gene among our studied isolates.
- Carba NP strip test can offer a rapid and reliable alternative to other tedious techniques for the diagnosis of Carbapenemase production.

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