Comparison of Three Phenotypic Methods and PCR for Rapid Detection of Carbapenemase Production in *Klebsiella* spp. and *Acinetobacter* spp.

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Background and study aim: The last decade was marked by the emergence and spread of carbapenemases. The study aims to compare three phenotypic methods with PCR for rapid detection of carbapenemase in carbapenem resistant gram negative bacteria.

Material and Methods: This study was conducted on 50 carbapenem-resistant gram negative bacteria. clinical isolates in Central Microbiology Laboratory, Ain Shams University Hospitals. All isolates were tested for carbapenemase encoding genes (blaKPC, blaIMP, blaOXA-48, blaVIM and blaGES) using PCR. Phenotypic detection of carbapenemases was made by chromID® CARBA SMART Agar (CARB/OXA), Modified Hodge test and Blue-Carba test.

Results: The 50 isolates included *Klebsiella* spp. (28/50, 56%) and *Acinetobacter* spp. (22/50, 44%). All *Klebsiella* isolates were positive for one or more genes coding for carbapenemase by PCR, with predominance of blaOXA-48 gene, while 20/22 (90.9%) of *Acinetobacter* isolates were positive for one or more genes coding for carbapenemase by PCR with predominance of blaOXA-48 gene. Chromogenic media gave positive results in 98% (49/50) of isolates with sensitivity 100% and specificity 0% for detection of carbapenemase producers. MHT gave positive results in 45 isolates out of 48 PCR positive isolates with a sensitivity of 93.8% and specificity 0% for detection of carbapenemase production. Blue carba test gave positive results in 48 isolates out of 48 PCR positive isolates with a sensitivity of 100% and specificity 100% for detection of carbapenemase production.

Conclusion: Blue carba test agreed with PCR with a sensitivity of 100% and specificity 100%, However it failed to detect the carbapenemases detected by other phenotypic tests .

INTRODUCTION

The most common bacterial isolates identified from clinical specimens are Gram-negative bacilli belonging to the Enterobacteriaceae family. Entero-bacteriaceae are responsible for roughly 70% of urinary tract infections, 50% of septicemia cases, and a large percentage of intestinal infections **[1]**. Antibiotic resistance genes, such as extended-spectrum Beta-lactamases (ESBLs), AmpCs, and carbapenemases, can be acquired by these bacteria **[2]**.

Multidrug resistance is spreading rapidly among a wide range of bacterial species, resulting in community-acquired and nosocomial illnesses **[3]**. Carbapenems, the last line of treatment, are routinely used to treat nosocomial infections, and rising resistance to this family of ß-lactams leaves us with fewer options.

Carbapenem resistance in Gram-negative bacteria has become a major public health issue. Production of Beta-lactamases, porin loss, Penicillin-binding protein change, and/or overexpression of efflux systems have all been proposed as mechanisms of carbapenem resistance **[4]**. In Gram-negative bacteria, carbapenemase synthesis is the most significant mechanism of carbapenem resistance. The appearance and dissemination of carbapenemases like K. pneumoniae carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), and Oxacillinase-

48 (OXA-48) among Enterobacteriaceae isolated globally highlighted the recent decade **[5]**.

For a variety of reasons, accurate detection of carbapenemase synthesis is critical. The most important goal is to direct infection control resources and treatments **[6]**. Colorimetric techniques for detecting carbapenemase production directly in bacterial isolates recovered from microbiological cultures (e.g. Carba NP and Blue Carba tests) are simple to use and reliable enough to be employed in normal laboratory work. They are based on the enzymatic hydrolysis of a carbapenem's Beta-lactam ring (usually imipenem), which results in the acidification of an indicator solution, which changes colour owing to pH change. Using colorimetric techniques for detection of carbapenemase production in *Enterobacteriaceae* (CPE) takes up to 2 hours **[7]**.

The modified Hodge test (MHT) is a phenotypic test used to screen for carbapenemase producers microorganisms. This test is based on carbapenemase-producing microorganisms inactivating a carbapenem, allowing a sensitive indicator strain to grow toward a disc containing this antibiotic, along the streak of inoculum of the tested strain. The MHT has showed high sensitivity in detecting carbapenemase producers from classes A and D. Unfortunately, the MHT has a limited sensitivity for detecting NDM-producing isolates, with a sensitivity of less than 50% **[8]**. The study aims to compare three phenotypic methods with PCR for rapid detection of carbapenemase in carbapenem-resistant gram negative bacteria (*Klebsiella*, and *Acinetobacter*).

MATERIALS AND METHODS

This study was conducted in Central Microbiology Laboratory, Ain Shams University Hospitals. Various clinical samples were cultured on blood agar plate (Himedia, India) and MacConkey agar plate (Himedia, India). Isolates were identified using Vitek 2 compact system (BioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing was done for gram negative isolates using disk diffusion method to detect resistance to Imipenem or Meropenem **[9]**. Sixty gram negative Imipenem or Meropenem resistant isolates were collected. These isolates were screened phenotypically for carbapenemases production by chromID® CARBA SMART Agar (CARB/OXA) (BIOMERIEUX, France) **[10]**, Modified Hodge test (MHT) **[11]** and Blue-Carba test **[12]**. These 50 isolates were preserved in glycerol tryptic soya broth in -80 C^0 for testing by PCR for carbapenemases genes (bla_{KPC}, bla_{IMP}, bla_{OXA-48}, bla_{VIM} and bla_{GES}) **[13]**.

Phenotypic screening of carbapenemases

A. ChromID® CARBA SMART Agar (CARB/OXA)

ChromID® CARBA SMART Agar (BIOMERIEUX, France) is made up of two chromogenic culture media dispensed onto a single Petri dish with two compartments (CARB/OXA). ChromID CARBA SMART Agar (CARB/OXA) is made out of a nutrient-dense foundation made up of several peptones. It comprises a cocktail of antibiotics that allows KPC and metallo-carbapenemase-type CPE to develop selectively in the CARB medium and OXA-48-type CPE in the OXA medium. It also includes three chromogenic substrates that may be used to identify the most often isolated CPEs. Escherichia coli produces spontaneous coloration (pink to burgundy) in strains producing ß-glucuronidase (ßGUR) and/or ß-galactosidase (ß-GAL), while Klebsiella, Enterobacter, Serratia, Citrobacter (KESC) produces spontaneous coloration (bluish-green to bluish-grey or purple) in strains producing ß-glucosidase (ß) and *Acinetobacter spp* produces no color [10]. Isolates were streaked on chromID® CARBA SMART Agar (CARB/OXA) (BIOMERIEUX, France) once on each side of the plate. The bacterial growth and color change of colonies were observed.

B. Modified Hodge test (MHT)

In brief, a lawn of 0.5 McFarland suspension of carbapenem susceptible strain E. coli ATCC 25922 was streaked over Mueller Hinton agar (MHA) plates (Himedia, India), and a meropenem antibiotic disc (10 ug) (Oxoid, UK) was inserted in the center of the MHA agar plate. The isolates were streaked in straight lines from the edge of the meropenem disc to the edge of the plate, together with a positive control (K. pneumoniae ATCC1705) and a negative control (K. pneumoniae ATCC1706). Plates were inoculated and allowed to dry at room temperature for 15 minutes before being incubated for 16–24 hours at 35 ± 2 ^{C}. When a clover leaf-like form was observed, the test was considered positive **[11]**.

C. Blue-Carba test

The Blue-Carba test (BCT) is a biochemical test for detecting carbapenemase synthesis in Gram-negative bacteria directly from bacterial culture in as short as two hours. It is based on bacterial colonies hydrolyzing imipenem in vitro (direct inoculation without previous lysis), which is identified by changes in pH values given by the indicator bromothymol blue **[7]**.

The test solution consisted of an aqueous solution of bromothymol blue at 0.04% (El-Nasr pharmaceutical chemicals company, Egypt) adjusted to pH 6.0, 0.1 mmol/liter ZnSO4 (El-Nasr pharmaceutical chemicals company, Egypt), and 3 mg/ml of imipenem powder (Merck & Co, USA), with a final pH of 7.0. A negative-control solution (0.04% bromothymol blue solution, pH 7.0) was prepared to control the influence of bacterial components or products on the pH of the solution. In 96- well round bottom microtiter plate (CITOTEST, Haimen, China) a 100µL of imipenem solution were added in each well of the plate in addition to 50µL of Zn sulphate solution and 50µL of bromothymol blue solution. A loop (approximately5 µl) of a pure bacterial culture recovered from Mueller-Hinton agar (Himedia, India) was directly suspended in 200 µl of both test and negative-control solutions in the 96-well microtiter plate and incubated at 37°C. Carbapenemase activity was revealed when the test and negative-control solutions, respectively, were (i) yellow versus blue, (ii) yellow versus green, or (iii) green versus blue. Non-carbapenemase producers remained blue or green on both solutions. The test was performed in triplicate for all isolates, yielding reproducible results **[12]**.

Genotypic detection of carbapenemases genes by real time PCR

Total DNA was extracted using Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, Dreieich, Germany) according to manufacturer's instructions. Amplification was performed using Maxima SYBER Green qPCR Master Mix (2x) (Thermo Scientific, Germany) and primers for amplification of (bla_{KPC}, bla_{GES}, bla_{OXA-48}, bla_{IMP} and bla_{VIM}) (table 1) **[13]** genes were used in a total volume of 25 µl. Each gene was run separately at a time. The amplification was carried out on Rotor-Gene Q (Qiagen, Germantown, USA) thermal cycler **[14]**.

Statistical methods

The collected data were computerized and statistically analyzed using the Statistical Package for Social Sciences (SPSS 24 Inc. Chicago, IL, USA). Chi-Square test was utilized to inspect the association between two qualitative variables (count >5). Fisher's exact test was implemented to evaluate the association between two qualitative variables (count < 5). A p-value < 0.05 was considered statistically significant.

Table (1): Primers used for real time PCR **[13]**

	Forward Primers
bla_{KPC}	5`TCGCTAAACTCGAACAGG
bla_{GES}	5`CTATTACTGGCAGGGATCG
bla_{OXA-48}	5`TGTTTTTGGTGGCATCGAT
bla_{IMP}	5`GAGTGGCTTAATTCTCRATC
bla_{VIM}	5`GTTTGGTCGCATATCGCAAC
	Reverse Primer
bla_{KPC}	5` TTACTGCCCGTTGACGCCCAATCC
bla_{GES}	5` CCTCTCAATGGTGTGGGT
bla_{OXA-48}	5` GTAAMRATGCTTGGTTCGC
bla_{IMP}	5` AACTAYCCAATAYRTAAC
bla_{VIM}	5` AATGCGCAGCACCAGGATAG

Supplementary table 1

	bla$_{KPC}$	**bla$_{VIM}$**	**bla$_{OXA-48}$**	**bla$_{GES}$**	**bla$_{IMP}$**
1. klebseilla			●	●	
2. klebseilla	●	●	●	●	
3. klebseilla		●	●	●	
4. klebseilla		●		●	
5. klebseilla	●	●	●		
6. klebseilla		●	●	●	
7. klebseilla			●		
8. klebseilla			●		
9. klebseilla	●	●	●	●	
10. klebseilla			●	●	
11. klebseilla		●	●		
12. klebseilla	●	●	●	●	
13. klebseilla		●	●	●	
14. klebseilla	●	●	●		
15. klebseilla		●	●		
16. klebseilla			●	●	
17. klebseilla	●	●	●	●	
18. klebseilla		●	●		
19. klebseilla		●	●		
20. klebseilla	●	●	●		
21. klebseilla		●	●	●	
22. klebseilla	●	●	●		
23. klebseilla	●	●	●		
24. klebseilla	●	●	●		
25. klebseilla		●	●		
26. klebseilla	●	●		●	
27. klebseilla		●	●		
28.klebseilla	●	●			
29. Acinetobacter		●	●	●	
30. Acinetobacter			●		
31. Acinetobacter		●	●	●	
32. Acinetobacter		●	●		
33.Acinetobacter		●	●	●	
34. Acinetobacter		●	●		
35. Acinetobacter	●	●	●	●	
36. Acinetobacter			●	●	●
37. Acinetobacter		●	●	●	
38. Acinetobacter		●	●		
39. Acinetobacter		●	●		
40. Acinetobacter			●	●	
41. Acinetobacter		●	●	●	
42. Acinetobacter		●	●	●	
43. Acinetobacter			●		
44. Acinetobacter	●	●			
45. Acinetobacter			●		●
46. Acinetobacter		●	●	●	
47. Acinetobacter		●		●	
48. Acinetobacter		●		●	
49. Acinetobacter		●	●		
50. Acinetobacter			●		

RESULTS:

In the current study, out of 60 gram negative Imipenem or Meropenem resistant isolates, there were 50 isolates carbapenemase producers (50/60, 83.3%). They included *Klebsiella spp*. (28/50, 56%) and *Acinetobacter spp.* (22/50, 44%). The 50 isolates were genotypic tested by real time PCR for (bla$_{KPC}$, bla$_{IMP}$, bla$_{OXA-48}$,

bla_{VIM} and bla_{GES}) genes with 48/50 (96%) isolates were positive to one or more of the tested genes coding for carbapenemase production, and 2/50 (4%) isolates were negative for these genes. All *Klebsiella* isolates 28/28 (100%) were positive for genes coding for carbapenemase by PCR, with predominance of bla_{OXA-48} gene (table 2) while *Acinetobacter* isolates 20/22 (90.9%) were positive for genes coding for carbapenemase by PCR with predominance of bla_{OXA-48} gene (table 2).

As regard ChromID® CARBA SMART Agar (CARB media), in the current study, all *Klebsiella* isolates grew on CARB media 28/28 (100%) and showed bluish green colonies. While 21/22 (95.5%) of *Acinetobacter* isolates grew on CARB media with no color and 1 isolate (2%) failed to grow on CARB media.

As regard ChromID® CARBA SMART Agar (OXA media), in the current study, among *Klebsiella* isolates 14/28 (50%) grew on CHROM OXA media with positive blue color, while other 14 isolates failed to grow. Concerning *Acinetobacter* isolates, 13/22 (59.1%) grew on CHROM OXA media with no color, while 9/22 (40.9%) failed to grow including the one failed to grew on CARB media.

In the current study, Comparing results of ChromID® CARBA SMART Agar and PCR showed 47/50 (94%) of positive isolates by chromogenic media were also positive by PCR, while only 2 positive isolates by chromogenic media were negative by PCR. Negative PCR isolates may carry another genes code for carbapenemases which are not included in our study and this may explain the cause of negative isolates by PCR which grew on positive ChromID® CARBA SMART Agar. There was highly statistical significance between results of ChromID® CARBA SMART Agar and real time PCR (P value, 0.001) (table 3). Diagnostic Performance of Chromogenic media were 100% sensitivity, specificity 0%, PPV 96%, NPV 0% for detection of carbapenemase production (Table, 6).

In the current study, Concerning Modified Hodge test, forty seven out of fifty isolates were positive by MHT, while 3 isolates were negative. All *Klebsiella* isolates 28/28 (100%) were positive, while 19/22 (86.4%) *Acinetobacter* were positive and 3/22 (13.6%) were negative. There was non- statistical significance between MHT and PCR (P value, 0.7) (table 4). Forty five isolates were positive by both MHT and PCR for carbapenemases, while three positive PCR isolates were negative by MHT and two isolates showed false positive results by MHT. Diagnostic Performance of MHT were 93.8% sensitivity and specificity 0% for detection of carbapenemase production (table 6).

In the current study, there were 48/50 (96%) isolates positive by blue carba test, while 2/50 (4%) were negative. Among the *Klebsiella* isolates 28/28 (100%) tested isolates were positive by blue carba test, while 20/22 (90.9%) *Acinetobacter* isolates were positive. There was highly-significant statistical association (***P.*‹0.001**) between blue carba test and PCR as 48/48 (100%) positive by blue carba test were PCR positive and the 2/2 (100%) negative isolates by blue carba test were also PCR negative (table, 5). Diagnostic Performance of blue carba test was 100% sensitivity and specificity 100% for detection of carbapenemase production (table 6).

Table (2): Distribution of the tested genes among *Acinetobacter*, and *klebsiella.*

		Acinetobacter		***klebsiella***	
		N	**%**	**N**	**%**
bla_{KPC}	**Positive**	2	9 %	12	42.8%
	Negative	20	91%	16	57%
Bla_{VIM}	**Positive**	16	72.7%	23	82.4%
	Negative	6	27.2%	5	17.6%
Bla_{OXA}	**Positive**	19	86.3%	25	89%
	Negative	3	13.6%	3	10.7%
Bla_{GES}	**Positive**	12	54.5%	13	47.1%
	Negative	10	45.4%	15	53.5%
Bla_{IMP}	**Positive**	2	9%	0	0%
	Negative	20	91%	28	100%

Table (3): Results of chromogenic media in relation to PCR.

Chromogenic Media	PCR		Total	%	P.value	S
	Positive	Negative				
Positive	47(94%)	2(4%)	50	100%		
Negative	0(0%)	1(2%)	0	0		
Total	47	3	50	100%	‹ 0.001	H.S

Table (4): Result of MHT in relation to PCR.

MHT	PCR				Total	P.value	S
	Positive		Negative				
	N	%	N	%			
Positive	45	93.8%	2	100%	47		
Negative	3	6.3%	0	0%	3		
Total	48		2		50	0.7	N.S

Table (5): Results of blue carba test and PCR.

Blue Carba	PCR		Total	%	P.value	S
	Positive	Negative				
Positive	48	0	48	100%		
Negative	0	2	2	100%		
Total	48	2	50	100%	‹ 0.001	H.S

Table (6): Diagnostic Performance of all tests.

	Sensitivity	Specificity	PPV	NPV
MHT	93.8%	0 %	95.7%	0%
Chromogenic media	100%	0	96%	0
Blue carba	100%	100%	100%	100%

DISCUSSION

In the current study, All *Klebsiella* isolates 28/28 (100%) were positive for genes coding for carbapenamase by PCR, with predominance of bla_{OXA-48} gene while *Acinetobacter* isolates 20/22 (90.9%) were positive for genes coding for carbapenamase by PCR with predominance of bla_{OXA-48} gene. This is in accordance with **El-Kholy et al. [15]** who reported Carbapenem resistance was in 26.6–100% of A. baumannii, and 48.1–100% K. pneumoniae in Egypt. In the same study they found that Carbapenemase genes NDM and KPC were commonly reported in A. baumannii with a prevalence of 0–39.3% and 0–28.6%, respectively. In K. pneumoniae, there was a high prevalence of KPC, NDM and OXA-48 genes at 0–95.8%, 20.9–100%, and 0–80.65%, respectively.

In the current study, diagnostic Performance of Chromogenic media were 100% sensitivity, specificity 0%, PPV 96%, NPV 0% for detection of carbapenemase production. This was in agreement with **Vironi et al. [16]** and **Papadimitriou et al. [17]** who reported sensitivity (92.4%, 96.5% respectively) of ChromID® CARBA SMART Agar. **Simner et al. [18]** conducted another study in Canada, comparing five chromogenic media using 150 clinical isolates. The five chromogenic media evaluated were (i) Oxoid Brilliance ESBL (Besington, Hants, United Kingdom), (ii) Oxoid Brilliance CRE, (iii) bioMérieux chromID Carba (Marcy l'Étoile, France), (iv) CHROMagar Colorex C3Gr (Paris, France), and (v) CHROMagar Colorex KPC. They showed that ChromID Carba had the highest sensitivity of 89.9%.

In the current study, only 2 isolates positive by chromogenic media were negative by PCR, giving false positive results. In this context, **Aguirre et al. [6]** in Spain mentioned that false positive results of ChromID have been reported in strains harbouring ESBL or over expressing AmpC with porin deficiencies. This discrepancy could be explained as negative PCR isolates may carry another genes code for carbapenemases which are not included in our study and this may

explain the cause of negative isolates by PCR while gave positive results by chromogenic media.

In the current study, diagnostic Performance of MHT was 93.8% sensitivity and specificity 0% for detection of carbapenemase production. This is in agreement with **Mathers et al. [19]** in USA who studied 56 isolates using MHT and showed sensitivity results of 92.8%. Also, another study in USA by **Vasoo et al. [20]** worked on 131 clinical isolates that were positive for carbapenemase genes by PCR, and they found that sensitivity of MHT in detecting carbapenemase production compared to PCR was 98%. Also, **Creighton and Jayawardena [21]** in England who studied 50 isolates using MHT which gave sensitivity of 100%. On the other hand, **Doyle et al. [22]** in Canada studied 142 isolates using PCR, gave 69/142 positive MHT with overall sensitivity of 58% (sensitivity to metallo beta-lactamase, KPC, OXA-48 is 12%, 98%, and 93% respectively). Also, **Pasteran's et al. [8]** studied, in Argentina, 145 isolates positive for carbapenemase genes by PCR, sensitivity of MHT was 67%. **Martins et al. [4]** in Brazil mentioned that MHT is reliable in detection of KPC &OXA-48, but fails to identify metallo-beta lactamases and carbapenemases with poor hydrolytic activity this may explain why MHT gave false negative result in 3 isolates in the current study.

In the current study, two isolates were PCR negative while they are MHT positive which may be due to production of AmpC and ESBL combined to porin loss as mentioned by **CLSI [11], Lutgring and Limbago [23]** in USA and **Aguirre et al. [6]** in Spain. Also, the negative PCR isolates may carry another genes code for carbapenemases which are not included in our study and this may explain the cause of negative isolates by PCR which gave positive MHT [14].

In the current study, diagnostic Performance of blue carba test were 100% sensitivity and specificity 100% for detection of carbapenemase production. This is in agreement with **Novais et al. [24]** in Portugal who tested 75 isolates by blue carba and resulted in sensitivity (100%) compared to PCR results. Also **Pasteran et al. [7]** in Argentina studied 300 clinical isolates (188 known carbapenemase) using PCR. Comparing blue carba test to PCR, sensitivity of blue carba was 97 %, NPV was 96 %, specificity and PPV were 100%. Also, another study by **Dalmolin et al. [25]** in Brazil found that sensitivity of blue carba in detecting carbapenemases compared to PCR (100%) and specificity 100%. **Goudarzi et al. [26]** in Iran, reported sensitivity and specificity of blue carba in detecting carbapenemases approaching 100% and positive predictive values similar to our results. **Pires et al. [12]** in Portugal stated sensitivity and specificity of blue carba in detecting carbapenemases 100% as they performed the study on 101 clinical isolates. **Garcia-Fernandez et al. [27]** in Spain worked on 159 isolates. They compared blue carba test against PCR the gold standard, they found the sensitivity of blue carba in detecting carbapenemases and negative predictive value were 98% and 96% respectively that is matching with our research results [27]. Also**, Aguirre et al. [6]** in Spain revealed sensitivity of blue carba in detecting carbapenemases was 100% and specificity ranging 91-100%.

CONCLUSION

Blue carba test agreed with PCR with a sensitivity of 100% and specificity 100%, it failed to detect the carbapenemases detected by other phenotypic tests and not by PCR; and which may be due to carbapenemases other than blaKPC, blaVIM, blaIMP, blaGES and blaOXA tested in the current study.

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Conflict of interest: None.

Ethical considerations

All study procedures were in accordance with ethical guidelines of the 1975 Declaration of Helsinki and approved by the ethical committee of Faculty of Medicine, Ain Shams University.

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HIGHLIGHTS

- The current study aimed to compare three phenotypic methods with PCR for rapid detection of carbapenemase in carbapenem resistant gram negative bacteria.
- This study was conducted on 50 carbapenem-resistant gram-negative isolates that were isolated from different clinical samples in Central Microbiology Laboratory, Ain Shams University Hospitals. All isolates were tested

for carbapenemase production by PCR using (bla_{KPC}, bla_{IMP}, bla_{OXA-48}, bla_{VIM} and bla_{GES}) genes that code for common types of carbapenemases. Phenotypic detection of carbapenemases was made by chromID® CARBA SMART Agar (CARB/OXA), Modified Hodge test and Blue-Carba test.

- Our conclusion is that Blue carba test was positive in 48 isolates out of 48 PCR positive isolates with a sensitivity of 100% and specificity 100% for detection of carbapenemase production. Blue carba test is the best rapid method for detection of carbapenemase production in carbapenem resistant gram negative isolates.

REFERENCES

1. Kaye KS, Pogue JM. Infections Caused by Resistant Gram-Negative Bacteria: Epidemiology and Management. *Pharmacotherapy*. 2015; 35(10):949-62.
2. Vasoo S, Barreto JN, Tosh PK. Emerging issues in gram-negative bacterial resistance: an update for the practicing clinician. *Mayo Clin Proc*. 2015; 90(3):395-403.
3. Hrabák J, Chudáčková E, Papagiannitsis CC. Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. *Clin Microbiol Infect*. 2014; 20(9):839-53.
4. Martins WM, Cordeiro-Moura JR, Ramos AC, Fehlberg LC, Nicoletti AG, Gales AC. Comparison of phenotypic tests for detecting BKC-1-producing Enterobacteriaceae isolates. *Diagn Microbiol Infect Dis*. 2016; 84(3):246-8
5. Nordmann P, Poirel L. Strategies for identification of carbapenemase producing *Enterobacteriaceae*. *J Antimicrob Chemother. 2013*; 68(3):487–9.
6. Aguirre-Qui~nonero A and Martínez-Martínez L. Non-molecular detection of carbapenemases in *Enterobacteriaceae* clinical isolates. *J Infect Chemother. 2017;* (23):1-11.
7. Pasteran F, Veliz O, Ceriana P, Lucero C, Rapoport M, Albornoz E, et al. ReLAVRA Network Group. Evaluation of the Blue-Carba test for rapid detection of carbapenemases in gram-negative bacilli. *J Clin Microbiol*. 2015; 53(6):1996-8.
8. Pasteran F, Gonzalez LJ, Albornoz E, Bahr G, Vila AJ, Corso A. Triton Hodge Test: Improved Protocol for Modified Hodge Test for Enhanced Detection of NDM and Other Carbapenemase Producers. J Clin Microbiol. 2016; 54(3):640-9.
9. CLSI (Clinical Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing. 26th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2016. CLSI supplement M 100-S.
10. Panagea T, Galani I, Souli M, Adamou P, Antoniadou A, Giamarellou H. Evaluation of CHROMagar™ KPC for the detection of carbapenemase-producing Enterobacteriaceae in rectal surveillance cultures. *Int J Antimicrob Agents*. 2011; 37(2):124-8.
11. CLSI (Clinical Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing. 25th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2015. CLSI supplement M 100-S.
12. Pires J, Novais A, Peixe L. Blue-carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. *J Clin Microbiol*. 2013; 51(12):4281-3.
13. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. *J Antimicrob Chemother*. 2012; 67(4):906-9.
14. Meier M, Hamprecht A. Systematic Comparison of Four Methods for Detection of Carbapenemase-Producing *Enterobacterales* Directly from Blood Cultures. *J Clin Microbiol.* 2019; 23 : 57(11):e00709-19.
15. El-Kholy A, El-Mahallawy HA, Elsharnouby N, Abdel Aziz M, Helmy AM, Kotb R. Landscape of Multidrug-Resistant Gram-Negative Infections in Egypt: Survey and Literature Review. *Infect Drug Resist*. 2021; 14:1905-1920.
16. Vrioni G, Daniil I and Voulgari E. Comparative evaluation of a prototype chromogenic medium (ChromID CARBA) for detecting carbapenemase-producing Enterobacteriaceae in surveillance rectal swabs. *J Clin Microbio*. 2012; 50: 1841-1846.
17. Papadimitriou-Olivgeris M, Bartzavali C, Christofidou M, Bereksi N, Hey J, Zambardi G ,et al. Performance of chromID® CARBA medium for carbapenemases-producing Enterobacteriaceae detection during rectal screening. *Eur J Clin Microbiol Infect Dis*. 2014; 33(1):35-40.
18. Simner PJ, Gilmour MW, DeGagne P, Nichol K, Karlowsky JA. Evaluation of five chromogenic agar media and the Rosco Rapid Carb screen kit for detection and confirmation of carbapenemase production in Gram-negative bacilli. *J Clin Microbiol*. 2015; 53(1):105-12.
19. Mathers AJ, Carroll J, Sifri CD, Hazen KC. Modified Hodge test versus indirect carbapenemase test: prospective evaluation of a phenotypic assay for detection of Klebsiella pneumoniae carbapenemase (KPC) in Enterobacteriaceae. *J Clin Microbiol*. 2013; 51(4):1291-3.

20. Vasoo S, Barreto JN, Tosh PK. Emerging issues in gram-negative bacterial resistance: an update for the practicing clinician. *Mayo Clin Proc.* 2015; 90(3):395-403.
21. Creighton J and Jyawardena S. Comparison of four phenotypic tests, three biochemical tests and Cepheid Xpert® Carba-R for detection of carbapenemase enzymes in Gram-negative bacteria. *N Z J Med Lab Sci 2015; 69 (3):81*.
22. Doyle D, Peirano G, Lascols C, Lloyd T, Church DL, Pitout JD. Laboratory detection of Enterobacteriaceae that produce carbapenemases. *J Clin Microbiol*. 2012; 50(12):3877-80.
23. Lutgring JD, Limbago BM. The Problem of Carbapenemase-Producing-Carbapenem-Resistant-Enterobacteriaceae Detection. *J Clin Microbiol*. 2016; 54(3):529-34.
24. Novais Â, Brilhante M, Pires J, Peixe L. Evaluation of the Recently Launched Rapid Carb Blue Kit for Detection of Carbapenemase-Producing Gram-Negative Bacteria. *J Clin Microbiol*. 2015; 53(9):3105-7.
25. Dalmolin TV, Bianchini BV, Rossi GG, Ramos AC, Gales AC, Trindade PA, Campos MMA. Detection and analysis of different interactions between resistance mechanisms and carbapenems in clinical isolates of Klebsiella pneumoniae. *Braz J Microbiol*. 2017; 48(3):493-498.
26. Goudarzi H, Taherpour A, Fallah F, Pourkaveh B, Erfanimanesh S, et al. Laboratory Detection of Carbapenemases in Gram-Negative Bacteria. *Arch Clin Infect Dis*. 2016; 11(2):e32816.
27. García-Fernández S, Morosini MI, Gijón D, Beatobe L, Ruiz-Garbajosa P, Domínguez L, Cantón R, Valverde A. Detection of Carbapenemase Production in a Collection of Enterobacteriaceae with Characterized Resistance Mechanisms from Clinical and Environmental Origins by Use of Both Carba NP and Blue-Carba Tests. *J Clin Microbiol*. 2016; 54(2):464-6.