ORIGINAL ARTICLE

# Detection of *blaKPC* gene among Carbapenem Resistant Enterobacteriacae Isolates from Ain Shams University Hospital, Egypt

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***Key words:***
***Enterobacteriaceae, Carbapenem resistance, Antimicrobial susceptibility testing , blaKPC gene***

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## ABSTRACT

***Background:*** *Carbapenem resistance among Enterobacteriaceae, especially in Escherichia coli (E.coli) and Klebsiella pneumoniae (K. Pneumoniae) is considered a significant problem worldwide. Resistance to carbapenems in Enterobacteriaceae is mediated by different mechanisms. Production of class-A,* Klebsiella pneumoniae *carbapenemase (KPC) is the most common mechanisms.* ***Objective:*** *This work aims to measure the frequency of carbapenem resistance among enterobacteriacae isolates and detection of blaKPC gene among them.* ***Methodology:*** *Seventy (70) Enterobacteriaceae isolates were collected from Ain Shams University Hospital during a period of 3 months from February to May 2022.The bacterial isolates were identified by conventional methods. All Enterobacteriaceae isolates were screened for carbapenem resistance by disc diffusion method using meropenem disks. Twenty-five (25) CRE strains were tested for antimicrobial susceptibility testing by the KirbyBauer method. Then E test strips containing range of antibiotic concentrations (0.002-32 ug/ml) for meropenem was done for confirmation of CRE isolates. Twenty-five (25) CRE isolates were subjected to conventional PCR blaKPC gene detection.* ***Results:*** *Out of 70 Enterobacteriaceae isolates 36 strains were identified as K. pneumoniae, 25 were identified as E.coli and 9 were Proteus spp by conventional bacteriological methods. Twenty-five CRE isolates were detected by meropenem disk diffusion method (18 K. pneumoniae and 7 E.coli).. BlaKPC was detected in 3 out of CRE 22 isolates, (13.6%) by conventional PCR.* ***Conclusion:*** *CRE is increasing rapidly worldwide with emergence of BlaKPC gene carbapenem resistance.*

## INTRODUCTION

*Enterobacteriaceae* is a large family of Gram negative rods. *Enterobacteriaceae* are normally found in the intestinal tract of humans and animals[1]. There are more than 40 genera and 150 species included in this family. Only few are considered as true pathogens like (Escherichia, Salmonella, Shigella, and Yerssina), and few are opportunistic pathogens as (Klebsiella, Citrobacter, Enterobacter, Proteus, Morganella and *Serratia*)[1].

The existence of antimicrobial resistance among *Enterobacteriaceae* has been increasing and has become a major threat worldwide. Carbapenems (meropenem, doripenem, imipenem and ertapenem) are β-lactam broad spectrum antibiotics and are considered 'drugs of last treatment options for severe diseases caused by *Enterobacteriaceae*. Carbapenem-resistant Enterobacteriaceae (CRE) is increasing rapidly**.**

Resistance to Carbapenem is either due to production of carbapenem enzyme that breakdown carbapenem or production of beta-lactamase (ESBLs or Ampc) together with porin loss. CRE is multidrug-resistant organisms harboring resistance to most, if not all, commercially available antibiotics [3].Carbapenemase is classified to three classes molecularly A, B, and D βlactamase[4].

Class A carbapenemases are inhibited by clavulanic acid. Class B or metallo-βlactamases (MβLs) are inhibited by ethylene diamine tetra-acetic acid (EDTA) and class D oxacillinases which are not inhibited either by clavulanic acid or EDTA [5].

*K. pneumoniae* carbapenemase (KPC) belong to molecular class A , New Delhi metallo betalatamase (class B) and OXA48 (class D), are the most common carbapenemases produced by En*terobacteriaceae*[5]. These enzymes harbor resistance to all beta-lactam agents, including penicillins, cephalosporins, monobactams, and carbapenems [6]**.**

Rapid detection of CRE is crucial and important to clinical and public health. The construction of a simple laboratory method to detect CRE is mandatory. Carbapenem resistance can be detected phenotypically or by molecular techniques. Polymerase chain reaction

(PCR) is the gold standard and provides prompt method for the detection of CRE[7]. The aim of the work is to measure the frequency of carbapenem resistance among *enterobacteriacae* isolates and detection of *blaKPC* gene among them

## METHODOLOGY

**Identification of bacterial isolates:**

Seventy (70) *Enterobacteriaceae* isolates were collected from Ain Shams University Hospital during a period of 3 months from February to May 2022.

The bacterial isolates were identified by conventional methods according to Collee et al.[8]

Out of 70 *Enterobacteriaceae* isolates 36 were *K. pneumoniae*, 25 were *E.coli* and 9 isolates were *Proteus spp.*

**Detection of carbapenem resistance in *Enterobacteriaceae* isolates:**

All collected *Enterobacteriaceae* isolates were screened for carbapenem resistance by disc diffusion method using commercially prepared meropenem (MEM) (10µg) disks (Oxoid, England) and results were interpreted according to the recommendations of the CLSI[9]. until obtaining 25 CRE isolates.

Out of 36 *K. pneumoniae isolates*, 18 were carbapenem resistant, while among 25 *E.coli* isolates, 7 were carbapenem resistant to and no resistance was detected among *Proteus spp.*

**Antimicrobial susceptibility pattern in CRE strains:**

Antimicrobial susceptibility testing for the twenty-five(25) CRE isolates were done by the Kirby Bauer method , according to the guidelines of the Clinical and Laboratory Standards Institute[9].

The strains were subjected for susceptibility testing to the following antibiotics: Cefotaxime 30µg (CTX), Ampicillin 10µg (AM), Piperacillin /Tazobactam 110µg (TPZ), Amoxicillin/clavulanic acid 20/10µg (AMC), Sulphamethoxazole/Trimethoprim 25µg (SXT), Ciprofloxacin 5µg (CIP), Gentamycin 10µg (GN), Amikacin 30µg (AK), Aztreonam 30µg (ATM), Cefepime 30µg (FEP), Ceftazidime 30µg (CAZ), and Cefoxitine 30µg (FOX). *Escherichia coli* ATCC 25922 was used as a quality control strain for antimicrobial susceptibility testing obtained from the National Central Laboratory, Ministry of Health, Egypt.

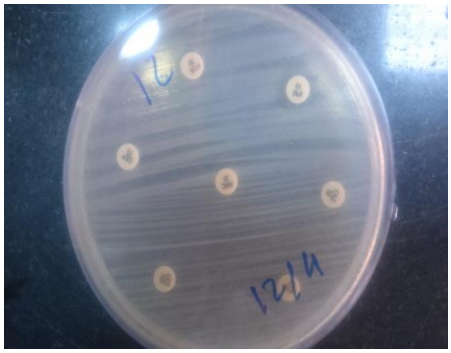

**Fig. 1:** A plate of Muller-Hinton agar inoculated by *k. pneumoniae* showing multidrug resistance**.**

**Determination of minimal inhibitory concentration of meropenem using Epsilometer test (E-test) for confirmation of CRE:**

Minimal inhibitory concentration (MIC) of meropenem was determined for CRE, by E-test method (Biomerieux, France) according to the manufacturer's instructions. E test strips containing range of antibiotic concentrations (0.002-32 ug/ml) for meropenem.

The values of the MIC of the antibiotic was interpreted according to CLSI as presented in table (1).

**Table 1: MIC values of antibiotics**

| **Antibiotic** | **MIC (ug/ml)** | | |
|---|---|---|---|
| **Meropenem** | **Sensitive** | **Intermediate** | **Resistant** |
| | ≤ 1 ug/ml | 2 | ≥4 ug/ml |

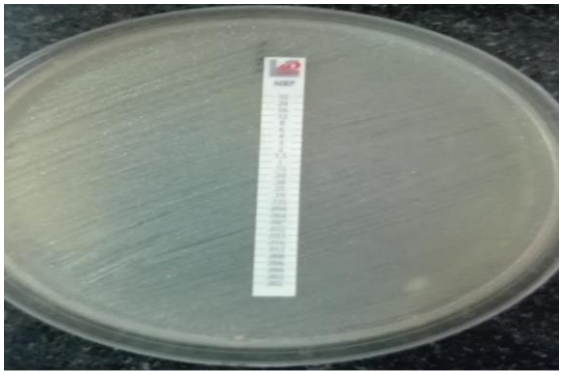

**Fig. 2:** Plate of CRE; Meropenem no zone of inhibition (resistant to meropenem)

**Molecular detection of carbapenems resistance gene *(blaKPC)_***

Twenty-five(25) CRE isolates CRE were tested by conventional PCR for detection of (*blaKPC*) carbapenemase gene

DNA extraction for the CRE bacterial isolates was performed using QIAGEN DNA extraction Kit® (QIAGEN, USA) following the manufacturer's instructions. Purified DNA was preserved at -20˚C until used. Then, amplification of class A carbapenemase (*blaKPC*) gene was done using primers supplied from Invitrogen as shown in Table (2). Polymerase chain reaction (PCR) was performed under the following conditions: 2 min at 50˚C, 10 min at 95˚C and 50 cycles of 15s at 95 ˚C and 1 min at 60˚C.The primer sequenece is shown in table 2.

**Table (2): Primer sequences of *blaKPC* used in this study**

| Gene | Primer Sequence | Amplified Product Size(bp) |
|---|---|---|
| *BlaKPC* -F | 5'GGC CGC CGT GCA ATA C3' | 890 ***(Rasheed et al., 2013)*** |
| *BlaKPC* -R | 5'GCC GCC CAA CTC CTT CA3' | |

**Statistical Analysis**:

Data were analyzed using a personal computer Statistical package for Social Science (SPSS 20) (Stat Soft Inc. USA).

## RESULTS

Out of 70 *Enterobacteriaceae*, *K. pneumoniae* were the most common isolated species , 36 (51.4%) followed by *E. coli* ,25 isolates of (35.7%) then 9 isolates of *Proteus spp* (12.9%) by conventional bacteriological methods. Twenty-five (35.7%) CRE isolates were detected by meropenem disk diffusion method (18 *K. pneumoniae* and 7 *E.coli*).

Table (3) and Fig (3) showed that 18/25 (72%) of isolated *K. pneumoniae* and 7 /25 (28%) of isolated *E.coli* were resistant to carbapenems while no resistant strains were detected among Proteus isolates.

**Table 3: The distribution of susceptible and resistant strains of *Enterobacteriaceae* isolates to carbapenems**

| *Enterobacteriaceae* | Sensitive | Resistant | Total |
|---|---|---|---|
| *K. pneumoniae* | 18(50%) | 18(50%) | 36(100%) |
| *E.coli* | 18(72%) | 7(28%) | 25(100%) |
| Proteus | 9(100%) | 0(0%) | 9(100%) |
| **Total** | **45(64.3%)** | **25(35.7%)** | **70(100%)** |

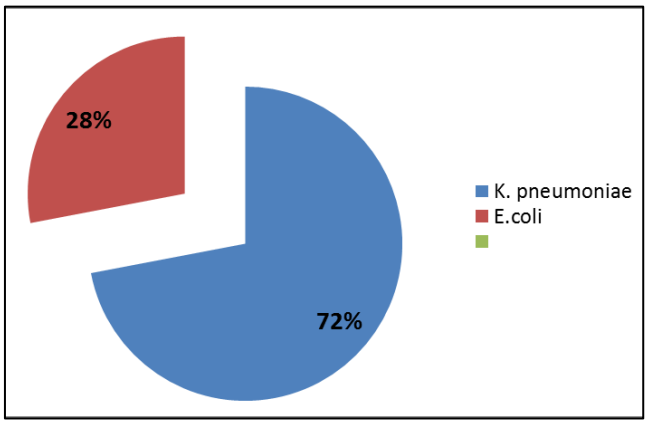


**Fig. 3:** Distribution of different CRE species

Table (4) shows that 13/25(52%) of CRE were susceptible to amikacin (AK). 4/25(16%) of CRE were susceptible to gentamycin (GN). 6/25(24%) were susceptible Ciprofloxacin (CIP). 1/25(4%) were susceptible Aztreonam (ATM) and Piperacillin /Tazobactam (TPZ). However 2/25(8%) were susceptible to sulphamoxazole trimethoprime (SXT) and Cefepime (FEP).

**Table (4): Antimicrobial susceptibility pattern of CRE isolates.**

| Antimicrobial | Sensitive | Intermediate | Resistant |
|---|---|---|---|
| **MEM** | 0(0%) | 0(0%) | 25(100%) |
| **AM** | 0(0%) | 0(0%) | 25(100%) |
| **ATM** | 1(4%) | 2(8%) | 22(88%) |
| **AMC** | 0(0%) | 0(0%) | 25(100%) |
| **AK** | 13(52%) | 0(0%) | 12(48%) |
| **CAZ** | 0(0%) | 0(0%) | 25(100%) |
| **FOX** | 0(0%) | 0(0%) | 25(100%) |
| **FEP** | 2(8%) | 0(0%) | 23(92%) |
| **TPZ** | 1(4%) | 0(0%) | 24((96%) |
| **CTX** | 0(0%) | 0(0%) | 25(100%) |
| **STX** | 2(8%) | 0(0%) | 23(92%) |

**Detection of CRE isolates by E test**

Meropenem MIC results by the E-test ( Fig.4), a total of 22 (35.7%) isolates were resistant to meropenem and MICs ranged from 0.002 to 32 ug/ml. Meropenem resistant strains included 15 *K. pneumoniae* (15/22 ;68.2%) and 7 *E.coli* (7/22 ;*31.8%)* .

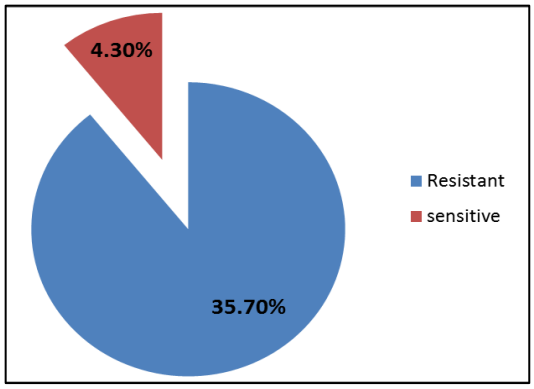


**Fig. 4:** Detection of CRE isolates by E-test

***Molecular detection of blaKPC gene* among CRE isolates**

PCR was performed to detect *blaKPC* expression. The gene was present in (3/22 isolates, 13.6%). The positive isolates were 3 *K. pneumoniae* (3/15 ;20%).

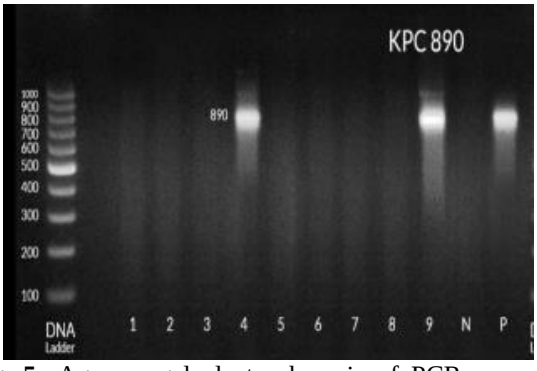


**Fig. 5:** Agarose gel electrophoresis of PCR-assay for detection of *blaKPC* gene. *BlaKPC* gene was detected at 890 bp

## DISCUSSION

Carbapenem is mainly used as last option in treatment of serious multidrug resistant infections. Early and effective detection of CRE is crucial for clinicians and infection preventionists to limit the spread of carbapenem-resistant among them[10]. CRE are of medical importance because of being plasmid-encoded carbapenemases and easily transferable.Spreading of these organisms may result in fatal infections with poor treatment outcome [11].

In our study *K.pneumoniae* were the commonest isolates (51.4%) followed by *E. coli* (35.7%) then *Proteus spp* (12.9%). This result with Hager et al.[12] who found that *K. pneumoniae* were the most common isolated CRE species (73.33%) followed by *E. coli* (26.67%). Also Zhang et al.[13] found that the most common organism was *K. pneumoniae* (73.9%), followed by *E. coli* (16.6%). Another study done in Egypt by El-Ghazzawy et al[14]. also reported that *K. pneumoniae* were the commonest isoltes (93.75%) followed by *E. coli* (3.75%).

The current study showed that the prevalence rate of CRE was 37.5% of *Enterobacteriaceae* isolates. Studies conducted in Egypt by Amer et al.[15] *and Mahmoud et al.*[16] found that 62.7% and 65.29% respectively were CRE. Mohamed et al.[17] in Egypt found that 19.9% of Enterobacteriaceae isolates were CRE.

The high prevalence rate of CRE could be explained by the misuse of antibiotics prior to result of culture and sensitivity. Amer et al.[15] in Egypt reported that the high level of resistance in his study may be due to unrestricted use of antibiotics which plays an important role in carbapenem resistance.

Other studies performed by Mohamed et al.[17]*also* reported that Eighteen (18) *K. pneumoniae* (76%) isolates and seven (7) *E.coli* (28%) out of twenty five CRE were resistant to carbapenem. This result agrees with Xu et al.[18] and Pang et al[19] , who reported that *K. pneumoniae* was the commonest CRE isolated. This disagreed with, Amjad et al.[20] who reported that *E-coli* was the commonest CRE.

In the current study 52% of CRE were susceptible to amikacin . 16% of CRE were susceptible to gentamycin. 24% were susceptible Ciprofloxacin. 4% were susceptible to Aztreonam and Piperacillin /Tazobactam. 8% were susceptible to sulphamoxazole trimethoprime and Cefepime. While, Ibrahim et al.[21] in Egypt reported that 40% of CRE isolates were susceptible to gentamycin, followed by amikacin and sulphamethoxazole trimethoprim, this is similar to results conducted in USA by Pollett et al.[22] and in Turkey by Baran and Aksu[23].

Regarding the meropenem MIC results by the E-test 35.7% isolates were resistant to meropenem .While another study done by Yu et al.[24] they reported that 96.6% of CRE isolates were resistant to meropenem E.test. Another study performed by Ibrahim et al.[21] showed that 88% of CRE isolates showed resistance to meropenem.

Regarding the results of PCR, 3 of the studied CRE isolates (13.6%) were positive for *blaKPC expression, including* 3 *K. pneumonia* (3/15; 20%).This report confirms with previously reported study by *Hager et al*[12].that found *blaKPC* was detected in 3 isolates (10%). However a study done by Metwally et al[25] detected a higher percentage, who found *blaKPC* in 14 out 20 (70%) of their isolates. And Melake et al.[26] reported *blaKPC* in 17.5% of CRE isolates.

Ongoing transfer of *blaKPC* gene may lead to higher rates of poor treatment outcomes ,thus increasing mortality rates. So further studies for CRE mediated by this gene should be conducted on greater collection of isolates.

## CONCLUSION

Resistance to carbapenem is increasing rapidly worldwide. *BlaKPC* gene is one of the important genes involved in the production of this resistance. Intervention should be in the form of early and rapid detection, good infection control practices and rationale use of antibiotics to ensure that the spread of drug resistance among bacteria is kept under control.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

**Ethical considerations:**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of interest**

All authors declare no conflict of interest in this work.

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## REFERENCES

1. Koneman EW, Allens SO, Janda WM, et al. Colour atlas and textbook for diagnostic microbiology. Enterobactericeae. JB Lippincott Company, Philadelphia; 2006. p. 61–402.
2. Riera E, Cabot G, Mulet X, García-Castillo M, del Campo R, Juan C, Canton R, Oliver A. *Pseudomonas aeruginosa* carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem and doripenem. J. Antimicrob. Chemother;2013,66(9):2022- 2027.
3. Centers for Disease Control and Prevention (CDC) .Vital signs: carbapenem-resistant Enterobacteriaceae. MMWR Morb Mortal Wkly Rep;.2013, 62:165-170.
4. Souli M, Galani I, Antoniadou A, et al. An outbreak of infection due to beta-lactamase *Klebsiella pneumoniae* carbapenemase-2 producing *K. pneumoniae* in a Greek University Hospital: molecular characterization,epidemiology, and outcomes. Clin Infect Dis. 2010;50(3):364–373.
5. Hammoudi D, Moubareck CA, Kanso A, Nordmann P, Sarkis DK. Surveillance of carbapenem non-susceptible Gram-negative strains and characterization of carbapenemases of classes a, b and d in a Lebanese Hospital. Leban. Med. J.;2015, 103(1973):1-8.
6. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, Quale J. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: A new threat to our antibiotic armamentarium. Arch. Int. Med.;2005, 165(12):1430-1435.
7. Lutgring JD, Limbago BM. The problem of carbapenemase producing carbapenem-resistant Enterobacteriaceae detection. J. Clin. Microbio;2016, 54(3):529-534. doi: 10.1128/JCM.02771-15.
8. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria (chapter 7). In: Collee J. G., Fraser, A. G. Marmion, B. P.1996.
9. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing. 32th. CLSI supplement M100S. Wayne, PA: CLSI, 2022.

10. Hu FP, Zhu DM, Wang F, Jiang XF, Xu YC, Zhang XJ, Zhang ZX, Ji P, Xie Y, Kang M, Wang CQ, Wang AM, Xu YH, et al. Report of CHINET Antimicrobial Resistance Surveillance Program in 2015. Chinese Journal of Infection and Chemotherapy. 2016; 16:685–694.
11. Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. Epidemiology of carbapenemase-producing Int. J. Appl. Microbiol. Biotechnol. Res. 93 Enterobacteriaceae and *Acinetobacter baumannii* in Mediterranean countries. BioMed. Res. Int. Doi;2014, 10.1155/2014/305784.
12. Hager R, Abdel El Salam SA, Abou El Magd NM. Emergence of carbapenem resistant enterobacteriacae coharboring New Delhi metallobeta-lactamase (NDM-1) and *Klebsiella pneumoniae* carbapenemase (KPC) isolated from a tertiary hospital in Egypt. Int. J. Appl. Microbiol. Biotechnol. Res 7; (2019) ,86-94.
13. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, Xie L et al. Epidemiology of carbapenem-resistant Enterobacteriaceae infections: Report from the China CRE Network. Antimicrob. Agents Chemother.;2018, 62(2):e01882-17.
14. El-Ghazzawy IF, Meheissen A, Younis DA. Phenotypic and genotypic methods for detection of metallo beta lactamases among carbapenem resistant Enterobacteriaceae clinical isolates in Alexandria Main University Hospital. Afr. J. Microbiol. Res.;2016, 10(1):32- 40.
15. Amer WH, Khalil HS, Abd EL Wahab MA. Risk factors, phenotypic and genotypic characterization of carbapenem resistant Enterobacteriaceae in Tanta University Hospitals, Egyptian International Journal of Infection Control. 2016; 12(2): 126-132. 20.
16. Mahmoud AB, Eissa NA, El-Raghy NA, El-Sebaey HM, Melake NA, Awad ET, Hamam SS. Phenotypic and molecular characterization of multidrug-resistant Enterobacteriaceae clinical isolates from intensive care units at Menoufia University hospitals. Menoufia Medical Journal. 2016; 29: 4: 835-845.
17. Mohamed T, Yousef L M, Darweesh E I, Khalil A H, Meghezel E M. Detection and Characterization of Carbapenem Resistant Enterobacteriaceae in Sohag University Hospitals. Egyptian Journal of Medical Microbiology. 2018; 27 (4): 61-69.
18. Xu Y, Gu B, and Huang M. Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000-2012 in Asia. Journal of Thoracic Diseases. 2015; 7: 376-385.
19. Pang F, Jia X, Zhao Q, Zhang Y. Factors associated to prevalence and treatment of carbapenem-resistant Enterobacteriaceae infections: a seven years retrospective study in three tertiary care hospitals. Annals of Clinical Microbiology and Antimicrobials. 2018; 17: 13. 8 pages.
20. Amjad A, Mirza IA, Abbasi SA, Farwa U, Malik N, and Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iranian Journal of Microbiology. 2011; 3: 189- 193.
21. Ibrahim DM, Taha SE Abdel El Salam SA, Abd El Aziz AA. Phenotypic Detection of Carbapenem Resistant Enterobacteriaceae, Isolates and Assessment of Their Susceptibility to the Novel Ceftazidime-Avibactam Combination. Egyptian Journal of Medical Microbiology.2019; 28(2):37-44.
22. Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM. Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. Journal of Clinical Microbiology. 2014; 52:4003–9.
23. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals of Clinical Microbiology and Antimicrobials 2016; 15(1):165-182.
24. Yu B, Dong D, Wang M, Guo Y, Yin D, Hu F. Evaluation of modified carbapenem inactivation method for suspected carbapenemase among Enterobacteriaceae clinical isolates. Oncotarget. 2018; 49(9): 29233-37
25. Metwally L, Gomaa N, Attallah M, Kamel N. High prevalence of *Klebsiella pneumoniae* carbapenemase-mediated resistance in *K. pneumoniae* isolates from Egypt. Eastern Mediterranean Health Journal.;2013, 19(11):947-952.
26. Melake NA, Mahmoud AB, Elraghy NA, Labib AZ, Hassan DM, Elbrolosy AM. Detection of *Klebsiella pneumoniae* carbapenemases and metallo-β-lactamases among *Klebsiella pneumoniae* isolates from hospitalized patients at Menoufia University Hospitals, Egypt. Menoufia Med. J.;2016, 29(4):801-811.