Microbes and Infectious Diseases 2024; 5(4): 1506-1518

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Phenotypic versus molecular assays for detecting carbapenemase-producing *Enterobacterales* from urinary tract infections

Yara Safwat Roshdy[1]*, Amina Mahmoud Nour El Deen[2], Yasmin Salah Naga[3], Ahmed Moustafa El Menshawy[4], Mai khaled Mahar[5]

1. Medical Microbiology and Immunology, Faculty of Medicine, Alexandria university, Egypt
2. Medical Microbiology and Immunology, Faculty of Medicine, University of Alexandria, Egypt.
3. Internal Medicine Nephrology Department , Faculty of Medicine, University of Alexandria, Egypt.
4. Lecturer in Critical care medicine, Faculty of Medicine, University of Alexandria, Egypt.
5. Assistant Lecturer in Medical Microbiology and Immunology, Faculty of Medicine, University of Alexandria, Egypt

ARTICLE INFO

Article history:
Received 3 July 2024
Received in revised form 28 July 2024
Accepted 5 August 2024

Keywords:
Carbapenem
β-lactamase
Carbapenemase
Antibiotic resistance
Enterobacterales

ABSTRACT

Background: Carbapenem-resistant *Enterobacterales* (CRE) have been identified as a public health problem. Treatment options for CRE are limited as they are mostly resistant to βeta-lactams, aminoglycosides, and fluoroquinolones as well as carbapenems. The present study aims to evaluate the performance of three phenotypic methods compared to a molecular-based technique for carbapenemase detection in *Enterobacterales* and determining their applicability in clinical and epidemiological settings. **Methods**: A total of 1,158 *Enterobacterales* were isolated from the urine samples in the microbiology laboratory of Alexandria main university hospital during the period from April 2020 to April 2021. Fifty randomly selected (39 *Klebsiella* and 11 *E. coli*) *Enterobacterales* were screened for carbapenem resistance by disc diffusion method. They were subjected to 3 phenotypic tests which are: Carba NP method, Modified carbapenem inactivation method (m CIM) and EDTA- modified carbapenem inactivation method (e CIM). Detection of 5 carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{OXA-48} and bla_{NDM-1}) was performed using real time PCR. **Results**: CRE represented 33% of *Enterobacterales* isolates. Twenty-six cases (52%) were males and 94% of the cases were above 40 years old. Carba NP test was positive in 43/50 (86%) of the selected isolates, m CIM was positive in 35/50 (70%) and e CIM was positive in 30/50 (60%). The most common carbapenemase gene detected was bla_{NDM-1} (94%), followed by bla_{OXA-48} gene (72%) and bla_{VIM} gene (24%). The bla_{KPC} gene and bla_{IMP} gene were not detected. Coexistence of the bla_{OXA-48} and the bla_{NDM-1} genes was detected in 48% isolates, while the bla_{NDM-1}, the bla_{OXA-48} and the bla_{VIM} genes were found in 22% isolates. The sensitivity of Carba NP, m CIM and e CIM was 87.5%, 72.9%, and 85.7% respectively. **Conclusion**: The study highlights the necessity of early detection of CRE. Carba NP test assists in the rapid identification of carbapenemase production. However, the genotypic test remains the gold standard for detection of CRE.

Introduction

Urinary tract infections are the third most frequent infection affecting humans after respiratory and gastrointestinal infections. They are an extremely prevalent cause of nosocomial infections in hospitalized patients [1]. According to The Infectious Diseases Society of America (IDSA) and

DOI: 10.21608/MID.2024.301393.2044
* *Corresponding author:* Yara Safwat Roshdy
E-mail address: *yara.safwat@alexmed.edu.eg*

the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), UTIs are classified into uncomplicated UTI and complicated UTI [2]. A complicated UTI includes: urinary tract infections in patients with urinary obstruction such as those presenting with stones, infections in immunosuppressed patients, Catheter-associated urinary tract infections (CAUTI), and those presenting with pyelonephritis or sepsis [3]. Indwelling urinary catheterization, which causes 40% of all hospital acquired infections worldwide, is the most common risk factor of complicated UTI [4].

E. coli is the most common bacterial agent causing uncomplicated UTIs followed by other *Enterobacterales* like *Klebsiella spp* and *Proteus spp* [5]. In comparison with uncomplicated UTIs, the microbes causing complicated UTIs are more likely to exhibit antimicrobial resistance [6]. The widespread use of empirical antibiotics leads to the spread of antibiotic-resistant genes. This contributes to the increased establishment of multidrug resistance (MDR) in bacteria making the treatment of UTIs more challenging [6]. The indiscriminate use of antibiotics has increased since the emergence of the COVID-19 pandemic which has led to the emergence of antibiotic-resistant organisms [7]. Resistant pathogens thrive in healthcare facilities, placing all patients at risk, regardless of their medical conditions [7].

Enterobacterales have evolved over time and developed different antibiotic resistance mechanisms aiding in their survival and multiplication [8]. The mainstays of today's antibacterial weapons against *Enterobacterales* continue to be β -lactams antibiotics [9]. The primary mechanism of beta-lactam resistance in *Enterobacterales* is the synthesis of beta-lactamases [10].

According to Ambler Classification, β-lactamases are divided into four classes from A to D based on the catalytic domain. Groups A, B, and D can also hydrolyze carbapenems (carbapenemases). [(11)] However, class C β-lactamases hydrolyze cephalosporins. Enzymes of classes A, C, and D contain serine in the active catalytic site, while class B enzymes have zinc in the active site [11].

In class A enzymes, the active serine group can be inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam [12]. On the other side, Metallo-beta-lactamase MBL (class B), which uses zinc ions to hydrolyze the βeta-lactam, can be inhibited by chelating agent as EDTA [12].

Class A carbapenemases include KPC (*Klebsiella pneumoniae* carbapenemase) which were first isolated from *K. pneumoniae* isolates in the United States, and afterwards they were reported from different sources across the world from other gram-negative microorganisms. Class A Carbapenemases are distinguished by their capacity to hydrolyze penicillins, cephalosporins, carbapenems, as well as aztreonam [13].

Class B carbapenemases, often known as metallo-beta-lactamase (MBL), are the most clinically relevant carbapenemases. This class of enzymes includes NDM (New Delhi metallo-β-lactamase), IMP (Imipenem-resistant Pseudomonas), and VIM (Verona integron-encoded metallo-β-lactamase). Oxacillin-hydrolysing carbapenemase (OXA) enzymes make up class D carbapenemases and OXA-48 is the most common in this class [14].

Carbapenemases genes are mostly found on plasmid vectors and other transposable elements, which allow their massive spread between bacteria [13,14].

The carbapenem-resistant *Enterobacterales* can be classified as: Carbapenemase-producing CRE (CP-CRE) and Non-carbapenemase-producing CRE (non-CP-CRE). CP-CRE are commonly plasmid -mediated leading to the horizontal transfer of one or more carbapenemase genes between different bacteria. [(15)] Non-CP-CRE are mainly caused by a combination of decreased outer membrane permeability together with efflux pump overexpression and production of beta-lactamases such as (AmpC-type or extended-spectrum-lactamases) [15].

The Centers for Disease Control and Prevention (CDC) modified the National Healthcare Safety Network (NHSN) surveillance definition for CRE to include *Enterobacterales* that showed carbapenemase production through a phenotypic or molecular assay or test resistant to any of the carbapenem agents, including ertapenem. This new definition may include non-CP-CRE [16].

As a result of the crucial connections between CP-CRE detection, prompt start of efficient antimicrobial medication, and infection control measures, the detection of CP-CRE and non-CP-CRE has grown to be of great significance. [(17)] Phenotypic and molecular assays for carbapenemase

production are essential for effective patient screening and management [17]. Some Phenotypic assays are colorimetric tests such as manual and commercial versions of the Carba NP [17]. Another category is the growth-based assays such as the Modified Hodge test [MHT] and the carbapenem inactivation method [CIM] [17]. Carbapenem hydrolysis-based assays using the MALDI-TOF-MS or spectrophotometric assays are another example. Immunochromatogenic tests have also been described [18].

Molecular assays have been the most successful method for studying the epidemiology of CRE and detecting carbapenemase genes. Molecular identification may be achieved in hours, allowing early diagnosis of CRE infections, initiating the appropriate treatments, and reducing the probability of infection spreading. However, this technology detects the existence of resistance genes but does not provide results of susceptibility testing or level of resistance gene expression. Furthermore, genetic variability in carbapenemase genes may limit molecular identification of CRE, leading in phenotypic resistance undetectable by gene sequence assays [19].

The aim of this study was to:

Evaluate the performance of three phenotypic methods compared to a molecular-based technique for carbapenemase detection in fifty clinical *Enterobacterales* isolates from urine samples of cases with urinary tract infection and determining their applicability in clinical and epidemiological settings.

Materials and methods

A total of 1,158 *Enterobacterales* isolated from the urine samples in the Microbiology laboratory of Alexandria main university hospital (AMUH) during the period from April 2020 to April 2021 were enrolled in the study. All the isolates were identified by conventional microbiological methods [20], followed by antibiotic susceptibility test (AST) according to CLSI 2020 (Ed30) guidelines using Bauer-Kirby disc diffusion technique. Screening for carbapenem resistance was carried out using ertapenem (ETP) 10 ug, meropenem (MEM) 10 ug, and imipenem (IPM) 10 ug discs [21].

Carbapenem resistant *Enterobacterales* showed reduced disc diffusion zone according to CLSI 2020 (Ed30): meropenem ≤22 mm, imipenem ≤22 mm and ertapenem ≤21 mm.

Fifty non-duplicated CRE isolates were randomly selected and were further subjected to 3 phenotypic tests which are:

- The Carba NP method
- The Modified carbapenem inactivation method.
- The EDTA- modified carbapenem inactivation method.

I. Phenotypic assays:

1. Carba NP method:

The test was done using the commercial RAPIDEC® CARBA NP Test (BioMérieux, La Balme-les-Grottes, France) following the manufacturer's Instructions. The RAPIDEC® CARBA NP test, which detects Carbapenem hydrolysis by Carbapenemase-producing bacteria, detects all 3 types of Carbapenemase. Hydrolysis acidifies the medium which results in the change in color of the pH indicator indicating the presence of Carbapenem resistance [22].

The test was done following the manufacturer's Instructions as follows:

a) Rehydration: 100 µL of the API suspension provided with the kit was pipetted into wells (a), (b) and (c) and was left for 10 minutes at room temperature (15-25°C).

b) Lysis: The contents of well (b) was mixed, CRE colonies picked from over-night-incubated blood agar plates was inoculated in well (c) until its turbidity matched well (b), followed by incubation for 30 minutes at room temperature (15-25°C), after bacterial lysis to extract the enzyme, the lysate was added to a detection solution which contains:(PH indicator, a carbapenem and zinc)

c) Hydrolysis: 25 uL from well (c) was transferred to wells (d) and (e) and 25 uL was transferred from well (a) to wells (d) and (e) and it was incubated for 30 minutes at 33-38°C.

d) Interpretation of Results:

If a color changed from red to yellow, light orange, orange or dark orange in well(e), the result was positive, and the test was complete.

If no color change was observed, the incubation at 33-38°C was continued for up to 2 hours before the final reading.

Modified Carbapenem inactivation method (m CIM):

The test was done according to the CLSI 2020 (Ed30) guidelines as follows [21]:

a) For each isolate to be tested, a 1-µL loopful of bacteria from an overnight blood agar plate were emulsified in 2 mL Tryptic Soy Broth (TSB). Followed by vortexing for 10–15 seconds.

b) 10-µg meropenem disk was added to each tube using sterile forceps making sure that the entire disk is immersed in the suspension.

c) It was then incubated at 35°C ± 2°C in ambient air for 4 hours ± 15 minutes.

d) A 0.5 McFarland suspension (using the colony suspension method) of E. coli ATCC® 25922 in nutrient broth was prepared immediately following completion of the TSB-meropenem disk suspension incubation.

e) The suspension was inoculated on Mueller Hinton Agar (MHA) plate as for the routine disk diffusion procedure making sure the inoculum suspension preparation and MHA plate inoculation steps are each completed within 15 minutes.

f) The meropenem disc was removed from each TSB-meropenem disk suspension using a 10-µL loop and was placed on the MHA plate previously inoculated with the meropenem-susceptible E. coli ATCC® 25922 indicator strain.

g) The MHA plates were inverted and incubated at 35°C ± 2°C in ambient air for 18–24 hours.

h) Following incubation, the zones of inhibition were measured as for the routine disk diffusion method.

Interpretation:

- Carbapenemase positive: Zone diameter of 6–15 mm or presence of pinpoint colonies within a 16–18 mm zone.
- Carbapenemase negative: Zone diameter of ≥ 19 mm (clear zone).
- Carbapenemase indeterminate: Zone diameter of 16–18 mm or zone diameter of ≥ 19 mm and the presence of pinpoint colonies within the zone.

EDTA- modified carbapenem inactivation method (e CIM):

The test was done according to the CLSI 2020 (Ed30) guidelines as follows [21]:

a) 20 µL of the 0.5 M EDTA was added to 2-mL TSB tube to obtain a final concentration of 5 mM EDTA.

b) Steps a through h were repeated as for m CIM procedure and processed the m CIM and e CIM tubes in parallel.

c) The meropenem discs from the m CIM and e CIM tubes were placed on the same MHA plate inoculated with the meropenem susceptible E. coli ATCC® 25922 indicator strain.

d) Interpretation:

- Metallo-beta-lactamase positive: ≥ 5-mm increase in zone diameter for e CIM vs. zone diameter form CIM.
- Metallo-beta-lactamase negative: ≤ 4-mm increase in zone diameter for the e CIM vs. zone diameter of m CIM.

Molecular testing:

The fifty CRE isolates were further analyzed for the presence of Five carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{OXA-48} and bla_{NDM-1}) using SYBR green real-time PCR.

DNA extraction:

- DNA for PCR was extracted using QIAMP DNA kit (Qiagen, Germany), according to the manufacturers' instructions. Evaluation of the quality of the extracted DNA was carried out using The NanoDrop Spectrophotometer (Thermo, USA).

Primers:

The primers used for PCR amplification of the different carbapenemase genes are shown in table 1.

PCR amplification:

a) The PCR reaction was performed in a total volume of 10 µL with a 4 µL DNA extract, 0.5 µL of each of the forward and reverse primers, 5 µL of master mix (DreamTaq Green PCR Master Mix, Thermo Scientific, USA). DreamTaq Green PCR Master Mix is a premixed solution containing DreamTaq DNA polymerase, optimized DreamTaq Green buffer, 4mM MgCl2 and 0.2 mM dNTPs. The amplification conditions were initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1.5 min, and a final elongation at 72°C for 7 min. Melting curve analysis was performed to check the specificity of the amplified products. The PCR products were electrophoresed and visualized under UV light to check specificity of the amplicons and the melting

temperature of each for further confirmation of the results (Figure 2).

b) The PCR reaction was done using the Rotor-Gene Q (Qiagen) real time PCR and was analyzed according to the melting temperature in the presence of positive and negative controls.

Statistical analysis

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The significance of the obtained results was judged at the 5% level.

Results

During the period from April 2020 to April 2021, a total of 1,158 *Enterobacterales* were isolated from the urine samples received in the microbiology laboratory of Alexandria main university hospital. *E. coli* represented the biggest number of isolates (572, 49%) of which 74 isolates (12.9%) were carbapenem resistant by disc diffusion. This was followed by *Klebsiella* (492,42%) isolates of which 309 (62.8%) were carbapenem resistant by the same screening method. *Enterobacter*, *Proteus* and *Citrobacter* species were isolated from a total of 94 (9%) urine samples, and they didn't show carbapenem resistance. Three hundred eighty-three (33%) isolates were carbapenem resistant *Enterobacterales* and 775 (67%) were carbapenem sensitive. *Klebsiella sp*. represented the majority of CRE (309 ,80.7%), versus (74, 19.3%) carbapenem resistant *E.coli*.

In the current study, 50 carbapenem resistant *Enterobacterales* were randomly selected (39 *Klebsiella* and 11 *E. coli*). Twenty nine out of 50 (58%) isolates were associated with CAUTI [22 *klebsiella* & 7 *E.coli*]. The remaining 21 isolates (42%) [17 *klebsiella* & 4 *E.coli*] were non CAUTI complicated UTI.

When analyzed according to the demographic data, it was found that 47 (94%) of the cases were above 40 years old. They were mostly residents of the internal medicine and ICUs while only 3 cases were from the urology department. Twenty-six cases (52%) were males and 24 (48%) were females. The Mean age of the patients was 62.66 ± 16.22. Complicated UTI was slightly higher in females (41.7%) than males (38.5%), while CAUTI was encountered more in males (61.5%) compared with females (58.3%), but this difference was not statistically significant (p 0.817).

Antimicrobial susceptibility test results

All the 50 isolates enrolled in the study were tested against 20 antimicrobial agents including ertapenem, imipenem and meropenem (Fosfomycin was tested with *E. coli* isolates only). The resistance rates of 39 *Klebsiella* and 11 *E. coli* isolates are shown in figure 2 and figure 3.

AMK: Amikacin, AMC: Amoxycillin/Clavulanic Acid, SAM: Ampicillin/Sulbactam, CIP: Ciprofloxacin, FEP: Cefepime, CAZ: Ceftazidime, CRO: Ceftriaxone, CXM: Cefuroxime, ETP: Ertapenem, GEN: Gentamycin, IPM: Imipenem, LVX: Levofloxacin, MEM: Meropenem, NIT: Nitrofurantoin, NOR: Norfloxacin, OFX: Ofloxacin, TZP: Piperacillin/Tazobactam, SXT: Trimethoprim/Sulfamethoxazole, CZA: Ceftazidime/Avibactam, COL: Colistin.

AMK: Amikacin, AMC: Amoxycillin/Clavulanic Acid, SAM: Ampicillin/Sulbactam, CIP: Ciprofloxacin, FEP: Cefepime, FOS: Fosfomycin, CAZ: Ceftazidime, CRO: Ceftriaxone, CXM: Cefuroxime, ETP: Ertapenem, GEN: Gentamycin, IPM: Imipenem, LVX: Levofloxacin, MEM: Meropenem, NIT: Nitrofurantoin, NOR: Norfloxacin, OFX: Ofloxacin, TZP: Piperacillin/Tazobactam, SXT: Trimethoprim/Sulfamethoxazole, CZA: Ceftazidime/Avibactam, COL: Colistin.

The results of the three phenotypic assays are shown in table 2.

Regarding the results of the genotypic test, 48 out of the 50 isolates (96%) were positive with one or more of the tested genes. The distribution of the five tested carbapenem resistance genes among all isolates is shown in table 3.

Correlating the results of the phenotypic tests (Carba NP, m CIM and e CIM) and molecular test, the Carba NP tested positive in 42/48 (87.5%) of the PCR positive isolates harboring one or more of the tested genes. Interestingly, only one out of 50 isolates were positive with the Carba NP but did not harbor any of the tested genes.

The m CIM tested positive in 35/48 (72.9%) of the PCR positive isolates harboring one or more of the tested genes. The e CIM tested positive in 30/48 (62.5%) of the PCR positive isolates. e CIM was positive in 29/47 (61.7%) of the isolates harboring metallo-beta-lactamases. Unexpectedly, one isolate was positive in the e CIM

but did not harbor any of the metallo-beta-lactamases tested in the present study.

The sensitivity, specificity, positive predictive and negative predictive values of the Carba NP in this study were 87.5%, 50%, 97.7% and 14.3% respectively considering that the genotypic test is the gold standard method. On the other hand, the sensitivity, specificity, positive predictive value and negative predictive value of the m CIM in this study were 72.9%, 100%, 100% and 13.3% respectively. The sensitivity of the e CIM to detect MBL production in this study was 85.3%.

Table 1. Sequences of primers used for detection of carbapenemase encoding genes in isolates of *Enterobacterales* by PCR

Primers	Sequence	Gene
KPC Forward [23]	5′-ATGTCACTGTATCGCCGTCT-3′	bla_{KPC}
KPC Reverse [23]	5′-TTTTCAGAGCCTTACTGCCC-3′	
IMP Forward [24]	5′-GAA GGY GTT TAT GTT CAT AC-3′	bla_{IMP}
IMP Reverse [24]	5′-GTA MGT TTC AAG AGT GATGC-3′	
VIM Forward [24]	5′-GTT TGG TCG CAT ATC GCA AC-3′	bla_{VIM}
VIM Reverse [24]	5′-AAT GCG CAG CAC CAG GATAG-3′	
OXA-48 Forward [23]	5'-TTGGTGGCATCGATTATCGG-3′	bla_{OXA-48}
OXA-48 Reverse [23]	5'- GAGCACTTCTTTTGTGATGGC-3'	
NDM-1 Forward [23]	5'-GGGCCGTATGAGTGA-3'.	bla_{NDM-1}
NDM-1 Reverse [23]	5'-GAAGCTGAGCACCGCATTAG-3'	

Table 2. Distribution of the isolates in the present study according to phenotypic tests' results

	Carba NP				**m CIM**				**e CIM**			
Organism	**positive**		**negative**		**positive**		**negative**		**positive**		**negative**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
E. coli (n=11)	8	72.7	3	27.3	8	72.7	3	27.3	7	87.5	1	12.5
Klebsiella (n=39)	35	89.7	4	10.3	27	69.2	12	30.8	23	85.2	4	14.8
Total	**43**	**86**	**7**	**14**	**35**	**70**	**15**	**30**	**30**	**85.8**	**5**	**14.2**

Table 3. The distribution of carbapenem resistance genes among the selected isolates

	NDM		**OXA 48**		**VIM**		**KPC**		**IMP**	
	NO.	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**
E. coli (n=11)	10	90.9	8	72.7	2	18.2	0	0	0	0
Klebsiella (n=39)	37	94.9	28	71.8	10	25.6	0	0	0	0
TOTAL n=50	47	94	36	72	12	24	0	0	0	0

Figure 1. Melting temperature curves of the bla_{OXA-48} (A), bla_{NDM-1} (B), and bla_{VIM} (C).

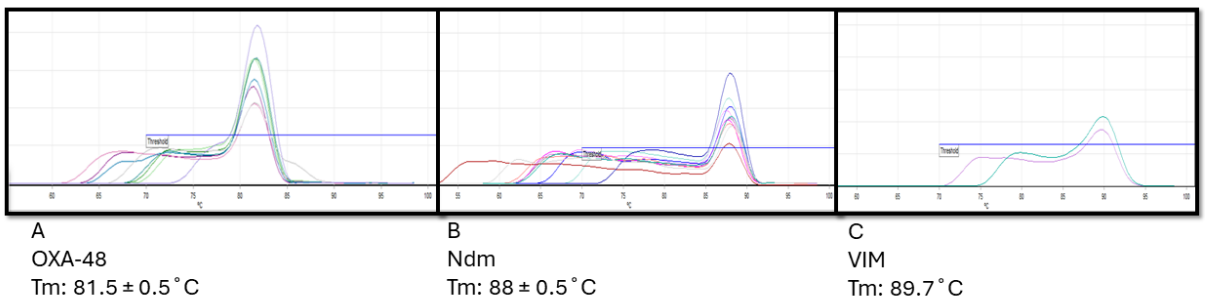


Figure 2. Antibiotic resistance rates of carbapenem-resistant *klebsiella* in the current study.

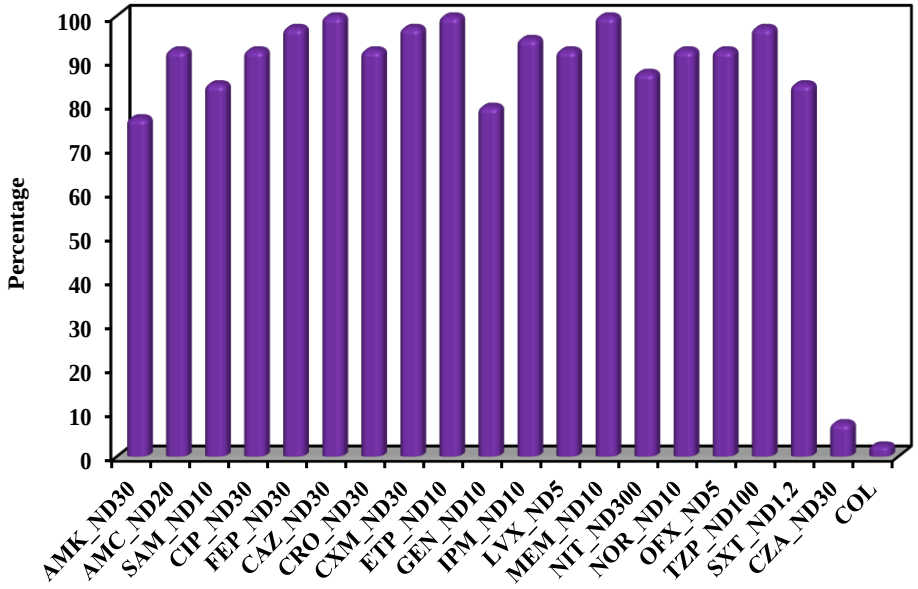
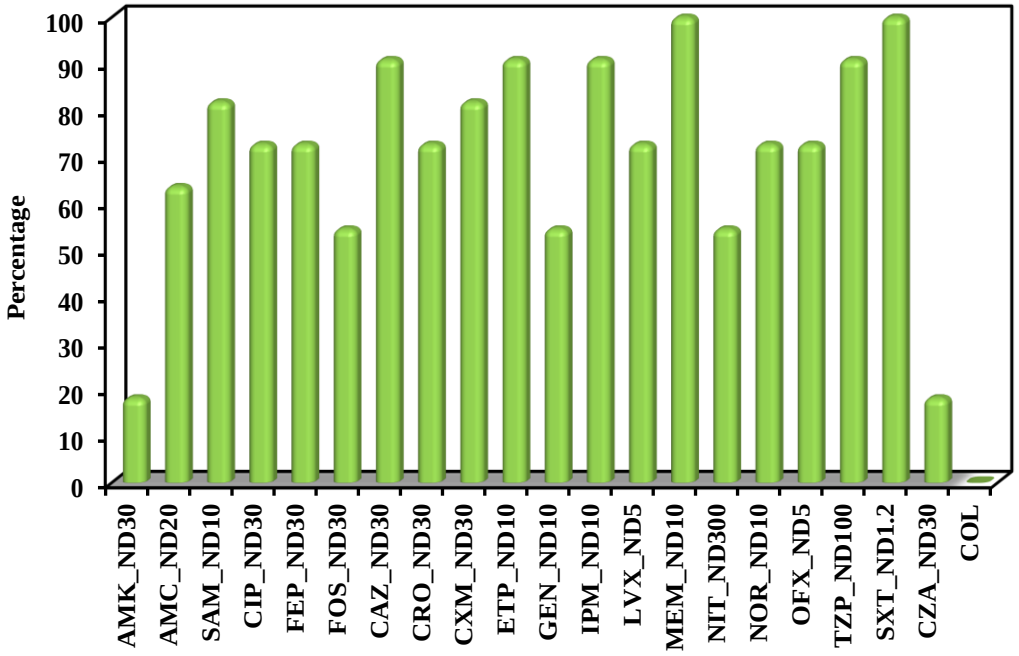


Figure 3. Antibiotic resistance rates of carbapenem-resistant *E. coli* in the current study.



Discussion

Carbapenem resistant *Enterobacterales* (CRE) are emerging as a significant contributor to health care-associated infections. Since treatment options are limited, timely detection of CRE is important [25]. The present study aimed to evaluate the performance of three phenotypic methods compared to a molecular-based technique for carbapenemase detection among *Enterobacterales*.

According to the present study, 33% of the isolates were resistant to carbapenems with higher prevalence of carbapenem resistant *Klebsiella sp*. (CRKP) (80.7%) compared with Escherichia *coli* (CREC) (19.3%). The current results are consistent with worldwide studies on the epidemiology of CRE which reported that the most prevalent carbapenem resistant *Enterobacterales* were *Klebsiella pneumoniae* (CRKP) and *Escherichia coli* (CREco) representing approximately 90% of all CRE isolates [26]. Moreover, our results agreed with studies conducted by Xu et al [27] and Pang et al [28] who

reported that *K. pneumoniae* was the most frequently isolated CRE [27,28].

Antimicrobial resistance expanded over the past two decades due to the augmented increase in global antibiotic consumption [17]. As a consequence, carbapenem-resistant *Enterobacterales* (CRE) endemic and epidemic emergence has been widely reported in hospitals [17]. Another important reason behind the wide spread of CRE across the globe is the exponential rise in antibiotic use during the (SARS-CoV-2) pandemic which aided in the selection of antibiotic-resistant bacteria, particularly carbapenemase producers [29]. SARS-CoV-2 pandemic exacerbated the antimicrobial resistance by increasing the risk of co-infection, unlicensed use of antibiotics, increasing the rate of empirical antimicrobial treatment for respiratory illness, decreasing resistance surveillance due to a focus on COVID-19 diagnosis [29].

According to the demographic data of our study, UTIs were slightly higher in males and this finding aligns with observations of previous studies [30,31]. However, the literature shows contradictory findings on the relevance of gender as a risk factor for drug-resistant UTIs [32]. While other research reported no differences at all regarding antibiotic sensitivity among *Enterobacterales* isolated from UTIs in males and females [32].

In the current study, the majority of CRE were isolated from patients with CAUTI (58%) and 50% of the isolates were mostly from ICUs. The high rate of CRE isolation from ICU may be explained by the longer hospitalizations, greater age of patients, greater antibiotic exposure and more carbapenem exposure, and required invasive therapy [33].

The antibiogram of the randomly selected 50 CRE isolates in the current study showed resistance to most of the antibiotics tested including cephalosporins, aminoglycosides and carbapenems. But almost all isolates were sensitive to colistin and ceftazidime-avibactam and this result is consistent with other studies [34,35]. Therefore, Combination therapy is recommended to lower both mortality and morbidity rates [36]. Contrary to our results, a high rate of colistin resistance among CRE was observed in a study by Armin S et al [37] and Haeili M et al [38].

In our study, Carba NP showed 87.50 % sensitivity and 50% specificity for detection of carbapenemase production. In agreement with our result, a previous study demonstrated excellent sensitivity of the Carba NP for most carbapenemases ranging from 73 to 100% [39]. On the contrary, lower rate was reported in a study in Iran where the Carba NP sensitivity was 25% [40].

The variance in Carba NP test sensitivity might be attributable to a range of factors, including changes in carbapenemase frequency rates, reduced hydrolyzing activity of some enzymes, decreased gene expression in some bacteria, and Mucoid colonies, which makes the protein extraction difficult [41].

The Carba NP specificity in this study (50%) was close to Thomson et al which showed that Rapidec Carba NP was 60.8% to 78.4% specific [42]. This may be explained by the ability of Carba NP to detect enzymes not encoded by the five tested genes [42]. In the current study, carba NP was negative in 6 isolates harboring either 2 or 3 carbapenemase genes including OXA-48. Similar findings were reported by Ho et al [43]. Ho et al. reported that they failed to detect more than two-thirds of the OXA-48 producing isolates using the Carba NP test [43]. The lower sensitivity and the limitation of Carba NP test to detect OXA-48 carbapenemases was explained by the limited hydrolytic activity of OXA-48 which may decrease the test sensitivity [44].

Regarding m CIM test diagnostic performance, our results were comparable to previous studies which have showed high sensitivity and specificity of mCIM for identifying widely detected carbapenemase types such as KPC, NDM, VIM, IMP, and OXA-48-like [45,46]. Consistent with our finding, Tsai YM et al reported similar specificity but higher sensitivity of m CIM (100%) [47]. Interestingly, The CIM test is known to have a decreased detection rates of the OXA-48-type carbapenemases, but this is not the case in the m CIM. However, it was mentioned in the CLSI that not all carbapenemase producing *Enterobacterales* are m CIM positive [21].

In our study, the sensitivity of the eCIM test for metallo-beta-lactamase detection (85.29 %) was comparable to those of Tsai et al. who reported 89.3% sensitivity of the eCIM [47]. Tsai et al showed a false-negative result by mCIM/eCIM although the presence of MBL in the isolate which showed low resistance to carbapenems. Tsai et al claimed that the carbapenem resistance level of

bacteria can affect the accuracy of mCIM/eCIM to detect carbapenemase, which necessitates further investigations [47].

Regarding the genotypic test results, 96% of the isolates were PCR positive. This result is comparable with literature as the sensitivities for molecular assays are between 97 and 100% [48]. Molecular methods continue to be most reliable and efficient for accurate carbapenemase detection [48].

In our study, the bla_{NDM-1} gene was the most prevalent (94%) followed by the bla_{OXA-48} gene (72%) and bla_{VIM} gene (24%) and this finding is consistent with a study conducted in Ain Shams University, Egypt [49].

Interestingly, high prevalence of concurrent multiple carbapenemases was detected in the current study with the coexistence of bla_{NDM-1} and bla_{OXA-48} as the most frequently detected combination and this finding is consistent with previous studies [50-52]. In the current study, neither bla_{KPC} nor bla_{IMP} was detected. Similarly, a study conducted in Kafrelsheikh, Egypt reported absence of bla_{KPC} and bla_{IMP} among their isolates [52].

The discovery of conjugative plasmids containing bla_{NDM-1} and bla_{OXA-48} genes in CRE isolates indicates that these plasmids aid in the spread of carbapenemase genes throughout *Enterobacterales* species. As a result, carbapenem resistance in CRE isolates is likely to be connected with the dissemination of these genes [53].

Interestingly in our study, one *klebsiella* isolate was PCR negative and Carba NP positive. The negative PCR result may be explained by the presence of other carbapenemases genes that were not included in our study [54].

Surprisingly, an *E.coli* isolate was negative for both phenotypic and genotypic tests in spite of being carbapenem resistant in antibiotic susceptibility test by disc diffusion. This can be explained by the possibility of the existence of a combination of other mechanisms of resistance e.g. (production of an ESBL or extended spectrum cephalosporinase combined with decreased bacterial cell wall permeability to influx of carbapenem antibiotics) [55].

Our study observed that the performance of genotypic tests is superior to the phenotypic tests, which has been supported by numerous prior studies utilizing molecular testing as the gold standard [48].

Conclusions

The results of this study showed a high carbapenem resistance rate among *Enterobacterales* (especially *Klebsiella spp*) isolated from urine cultures in our hospital settings, which reflects a threat limiting the treatment options in our hospitals. Most of the carbapenem resistant *Enterobacterales* (CRE) isolates in this study showed extensive drug resistance where they were only sensitive to colistin and ceftazidime-avibactam. Therefore, early detection of CRE is crucial for patient safety. The Rapidec® Carba NP test assists in the rapid identification of carbapenemase production. However, it is expensive and negative results should be confirmed with additional carbapenemase detection methods. The m CIM is cost-effective and easily adoptable on routine basis, but it requires overnight incubation. The m CIM together with e CIM are useful for identifying and classifying different types of carbapenemase in *Enterobacterales*.

The genotypic test remains the gold standard for detection of CRE. It is accurate and identifies the carbapenemase produced which is crucial for therapy, however it is expensive to be routinely used.

The bla_{NDM-1} gene was the most prevalent followed by the bla_{OXA-48}, their coexistence was frequently detected. Neither bla_{IMP} nor bla_{KPC} was detected in the present study.

Recommendations

The high percentage of carbapenem resistance calls for more rigid infection control measures and establishing strict antibiotic policies to limit the unnecessary use of carbapenems. Further studies are needed to investigate other phenotypic tests for detection of the type of carbapenemase produced.

Funding

None

Conflicts of interest

The authors declared no conflicts of interest.

References

1- Raoofi S, Pashazadeh F, Rafiei S, Hosseinipalangi Z, Noorani Z, Khani S et al. Global prevalence of nosocomial infection: A systematic review and meta-analysis. PLoS One 2023;18(1): e0274248.

2- Marantidis J, Sussman RD. Unmet needs in complicated urinary tract infections: Challenges, recommendations, and emerging treatment pathways. Infection and drug resistance 2023; 16:1391-405.
3- Wagenlehner FME, Bjerklund TE, Cai T, Koves B, Kranz J, Pilatz A, et al. Epidemiology, definition and treatment of complicated urinary tract infections. Nat Rev Urol 2020; 17(10):586-600.
4- Werneburg GT. Catheter-associated urinary tract infections: Current challenges and future prospects. Res Rep Urol 2022; 14:109-33.
5- Huang L, Huang C, Yan Y, Sun L, Li H. Urinary tract infection etiological profiles and antibiotic resistance patterns varied among different age categories: a retrospective study from a tertiary general hospital during a 12-year period. Front Microbiol 2022; 12:813145.
6- Wawrysiuk S, Naber K, Rechberger T, Miotla P. Prevention and treatment of uncomplicated lower urinary tract infections in the era of increasing antimicrobial resistance—non-antibiotic approaches: a systemic review. Arch Gynecol Obstet 2019; 300(4):821-8.
7- Malik SS, Mundra S. Increasing Consumption of Antibiotics during the COVID-19 Pandemic: Implications for Patient Health and Emerging Anti-Microbial Resistance. Antibiotics (Basel) 2022;12(1):45.
8- Spera AM, Esposito S, Pagliano P. Emerging antibiotic resistance: carbapenemase-producing enterobacteria. Bad new bugs, still no new drugs. Infez Med 2019; 27(4):357-64.
9- De Angelis G, Del Giacomo P, Posteraro B, Sanguinetti M, Tumbarello M. Molecular mechanisms, epidemiology, and clinical importance of β-lactam resistance in Enterobacteriaceae. Int J Mol Sci 2020; 21(14):5090.
10- Uc-Cachón AH, Gracida-Osorno C, Luna-Chi IG, Jiménez-Guillermo JG, Molina-Salinas GM. High prevalence of antimicrobial resistance among gram-negative isolated bacilli in intensive care units at a tertiary-care hospital in Yucatán Mexico. Medicina 2019;55(9):588.
11- Sawa T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance. J Intensive Care 2020; 8:13.
12- Lucic A, Malla TR, Calvopiña K, Tooke CL, Brem J, McDonough MA, et al. Studies on the Reactions of Biapenem with VIM Metallo β-Lactamases and the Serine β-Lactamase KPC-2. Antibiotics 2022; 11(3):396.
13- Hansen GT. Continuous evolution: perspective on the epidemiology of carbapenemase resistance among Enterobacterales and other Gram-negative bacteria. Infectious diseases and therapy 2021; 10.1: 75-92.
14- Reyes JA, Melano R, Cárdenas PA, Trueba G. Mobile genetic elements associated with carbapenemase genes in South American Enterobacterales. Braz J Microbiol 2020; 24: 231-8.
15- Ambretti S, Bassetti M, Clerici P, Petrosillo N, Tumietto F, Viale P, et al. Screening for carriage of carbapenem-resistant Enterobacteriaceae in settings of high endemicity: a position paper from an Italian working group on CRE infections. Antimicrob Resist Infect Control 2019; 8(1):136.
16- Lutgring JD. Carbapenem-resistant Enterobacteriaceae: an emerging bacterial threat. Semin Diagn Pathol 2019; 36(3):182-6.
17- Hussaini I, Suleiman A, Olonitola O, Oyi R. Phenotypic and molecular detection of carbapenemase producing Escherichia coli and

Klebsiella pneumoniae. Microbes and Infectious Diseases 2023; 4(1): 151-9.

18- Zhong H, Wu ML, Feng WJ, Huang SF, Yang P. Accuracy and applicability of different phenotypic methods for carbapenemase detection in Enterobacteriaceae: A systematic review and meta-analysis. J Glob Antimicrob Resist 2020;21:138-47.

19- Taggar G, Attiq M, Boerlin P, Diarra MS. Molecular Epidemiology of Carbapenemases in Enterobacterales from Humans, Animals, Food and the Environment. Antibiotics 2020; 9(10):693.

20- Altheide ST. Biochemical and Culture-Based Approaches to Identification in the Diagnostic Microbiology Laboratory. American Society for Clinical Laboratory Science 2020.

21- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests. 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

22- Hombach M, von Gunten B, Castelberg C, Bloemberg GV. Evaluation of the Rapidec Carba NP test for detection of carbapenemases in Enterobacteriaceae. J Clin Microbiol 2015; 53(12):3828-33.

23- Khan MA, Mohamed AM, Faiz A, Ahmad J. Enterobacterial infection in Saudi Arabia: First record of Klebsiella pneumoniae with triple carbapenemase genes resistance. J Infect Dev Ctries 2019; 13(04):334-41.

24- Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of Pseudomonas aeruginosa producing metallo-β-lactamases in a large centralized laboratory. J Clin Microbiol 2005; 43(7):3129-35.

25- Pecora ND, Li N, Allard M, Li C, Albano E, Delaney M, et al. Genomically informed surveillance for carbapenem-resistant Enterobacteriaceae in a health care system. MBio 2015; 6(4).

26- Ma J, Song X, Li M, Yu Z, Cheng W, Yu Z, et al. Global spread of carbapenem-resistant Enterobacteriaceae: Epidemiological features, resistance mechanisms, detection and therapy. Microbiol Res 2022; 266:127249.

27- Xu Y, Gu B, Huang M, Liu H, Xu T, Xia W, et al. Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000-2012 in Asia. J Thorac Dis 2015; 7(3):376-85.

28- Pang F, Jia XQ, Zhao QG, Zhang Y. Factors associated to prevalence and treatment of carbapenem-resistant Enterobacteriaceae infections: a seven years retrospective study in three tertiary care hospitals. Ann Clin Microbiol Antimicrob 2018; 17(1):13.

29- Moubareck C, Halat D. The Collateral Effects of COVID-19 Pandemic on the Status of Carbapenemase-Producing Pathogens. Front Cell Infect Microbiol 2022; 12:8236260.

30- A Lee DS, Choe HS, Kim HY, Yoo JM, et al. Role of age and sex in determining antibiotic resistance in febrile urinary tract infections. Int J Infect Dis 2016;51:89–96.

31- Rusu A, Tiliscan C, Adamescu AI, Ganea OA, Arama V, Arama SS, et al. Carbapenemase-producing uropathogens in real life: epidemiology and treatment at a County Emergency Hospital from Eastern Romania. J Med Life 2023 May;16(5):707-11.

32- McGregor JC, Elman MR, Bearden DT, Smith DH. Sex- and age-specific trends in antibiotic resistance patterns of Escherichia coli urinary isolates from outpatients. BMC Fam Pract 2013;14:25.

33- Kang JS, Yi J, Ko MK, Lee SO, Lee JE, Kim KH. Prevalence and Risk Factors of Carbapenem-resistant Enterobacteriaceae Acquisition in an Emergency Intensive Care

Unit in a Tertiary Hospital in Korea: a Case-Control Study. J Korean Med Sci 2019;34(18):e140.

34- Makharita RR, El-Kholy I, Hetta HF, et al. Antibiogram and Genetic Characterization of Carbapenem-Resistant Gram-Negative Pathogens Incriminated in Healthcare-Associated Infections. Infect Drug Resist 2020;13:3991-4002.

35- Chen J, Hu Q, Zhou P, Deng S. Ceftazidime-avibactam versus polymyxins in treating patients with carbapenem-resistant Enterobacteriaceae infections: a systematic review and meta-analysis. Infection 2024;52(1):19-28.

36- Aldali HJ, Khan A, Alshehri AA, Aldali JA, Meo SA, Hindi A, et al. Hospital-Acquired Infections Caused by Carbapenem-Resistant Enterobacteriaceae: An Observational Study. Microorganisms 2023;11:1595.

37- Armin S, Fallah F, Karimi A, et al. Antibiotic Susceptibility Patterns for Carbapenem-Resistant Enterobacteriaceae. Int J Microbiol 2023; 2023:8920977.

38- Haeili M, Javani A, Moradi J, et al. Alterations mediate colistin resistance in Klebsiella pneumoniae isolates from Iran. Frontiers in Microbiology 2017;8: 2470.

39- Kansak N, Çalık Ş, Arıcı N, Adaleti R, Aksaray S, Gönüllü N. Performance of the Rapidec® Carba NP assay for the detection of different carbapenemases in Klebsiella pneumoniae and Escherichia coli strains. Indian J Med Microbiol 2022; 40(4):516-20.

40- Armin S, Fallah F, Karimi A, Azimi T, Kafil H, Zahedani S, et al. Multicentre study of the main carbapenem resistance mechanisms in important members of the Enterobacteriaceae family in Iran. New Microbes New Infect 2021; 41:100860.

41- Fahim N, Elsayed L, ElMasry S. Diagnostic Efficacy of the Carba NP Strip Test for Carbapenemase Detection. Afro-Egyptian Journal of Infectious and Endemic Diseases 2023; 13(2), 80-9.

42- Thomson G, Turner D, Brasso W, Kircher S, Guillet T, Thomson K. High-stringency evaluation of the automated BD Phoenix CPO detect and Rapidec Carba NP tests for detection and classification of carbapenemases. J Clin Microbiol 2017; 55(12):3437-43.

43- Ho PL, Wang Y, Wing-Sze Tse C, et al. Rapid Detection of Carbapenemase Production in Enterobacteriaceae by Use of a Modified Paper Strip Carba NP Method. J Clin Microbiol 2017; 56(1):e01110-17.

44- Österblad M, Hakanen AJ, Jalava J. Evaluation of the Carba NP test for carbapenemase detection. Antimicrob Agents Chemother 2014; 58(12):7553-6.

45- Pierce VM, Simner PJ, Lonsway DR, et al. Modified Carbapenem Inactivation Method for Phenotypic Detection of Carbapenemase Production among Enterobacteriaceae. J Clin Microbiol 2017;55(8):2321-33.

46- Kuchibiro T, Komatsu M, Yamasaki K, et al. Evaluation of the modified carbapenem inactivation method for the detection of carbapenemase-producing Enterobacteriaceae. J Infect Chemother 2018;24(4):262-6.

47- Tsai YM, Wang S, Chiu HC, *et al.* Combination of Modified Carbapenem Inactivation Method (mCIM) and EDTA-CIM (eCIM) for Phenotypic Detection of Carbapenemase-Producing Enterobacteriaceae. BMC Microbiol 2020; 20: 315.

48- Rabaan AA, Eljaaly K, Alhumaid S, Albayat H, Al-Adsani W, Sabour AA. An Overview on

Phenotypic and Genotypic Characterisation of Carbapenem-Resistant Enterobacterales. Medicina (Kaunas) 2022;58(11):1675.

49- Shawky AM, Tolba S, Hamouda HM. Emergence of New Delhi Metallo Beta Lactamase blaNDM-1 and Oxacillinases blaOXA-48 Producing Klebsiella pneumoniae in an Egyptian Hospital. Egypt J Microbiol 2019; 54(1):25-37.

50- Raheel A, Azab H, Hessam W, Abbadi S, Ezzat A. Detection of carbapenemase enzymes and genes among carbapenem-resistant Enterobacteriaceae isolates in Suez Canal University Hospitals in Ismailia, Egypt. Microbes Infect Dis 2020; 1(1):24-33.

51- ElBaradei A. Co-occurrence of blaNDM-1 and blaOXA-48 among carbapenem resistant Enterobacteriaceae isolates causing bloodstream infections in Alexandria, Egypt. Egyptian Journal of Medical Microbiology 2022; 31(3): 1-7.

52- El-Domany R, El-Banna T, Sonbol F, Abu-Sayedahmed SH. Co-existence of NDM-1 and OXA-48 genes in Carbapenem Resistant Klebsiella pneumoniae clinical isolates in Kafrelsheikh, Egypt. Afr Health Sci 2021;21(2):489-96.

53- Solgi H, Badmasti F, Aminzadeh Z, Giske C G, Pourahmad M, Vaziri F, et al. Molecular characterization of intestinal carriage of carbapenem-resistant Enterobacteriaceae among inpatients at two Iranian university hospitals: first report of co-production of blaNDM-7 and blaOXA-48. Eur J Clin Microbiol Infect Dis 2017; 36: 2127–35.

54- Taher O. Comparison of Three Phenotypic Methods and PCR for Rapid Detection of Carbapenemase Production in Klebsiella spp. and Acinetobacter spp. Afro-Egyptian Journal of Infectious and Endemic Diseases 2022; 12(3): 236-44.

55- Ma J, Song X, Li M, Yu Z, Cheng W, Yu Z, et al. Global spread of carbapenem-resistant Enterobacteriaceae: Epidemiological features, resistance mechanisms, detection and therapy. Microbiol Res 2022; 266:127249.

Roshdy YS, Nour El Deen AM, Naga YS, El Menshawy AM, Mai khaled Mahar MK. Phenotypic versus molecular assays For detecting carbapenemase-Producing *Enterobacterales* From urinary tract infections. Microbes Infect Dis 2024; 5(4): 1506-1518.