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## EVALUATION OF THE PERFORMANCE OF ROUTINE PHENOTYPIC METHODS FOR ACCURATE AND RAPID DETECTION OF CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE* IN CLINICAL SAMPLES

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Ayman A. Farrag<sup>1</sup>, Mohamed Ali Abdel-Rahman<sup>1</sup>, Amani El-Kholy<sup>2</sup>, and Arwa R. Elmanakhly, Elsayed G.Salama<sup>1\*</sup>

<sup>1</sup> Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, P.N.:11884, Nasr City, Cairo, Egypt

<sup>2</sup> Department of Clinical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

<sup>3</sup> Microbiology and immunology Department, Faculty of pharmacy, Russian University in Cairo, Egypt.

\*Corresponding Author: SayedGamal999@gmail.com

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### ABSTRACT

Resistance to carbapenems among *Enterobacteriaceae* has emerged as a global threat, because carbapenems are considered the most potent antimicrobials used for treatment of severe Gram-negative infections. This study aimed to compare between the phenotypic and genotypic methods used for detection of carbapenemase-producing clinical isolates of carbapenem resistant *Enterobacteriaceae* (CRE). We evaluated modified Hodge Test (MHT), EDTA-imipenem combined disc test and modified carbapenem inactivation method (mCIM) in comparison with the multiplex polymerase chain reaction (PCR) method (gold standard) for detection of carbapenemase activity of 65 CRE isolates from patients admitted to a tertiary care hospital. The commonest source of CRE was blood culture (35%). The most common CRE type was *Klebsiella pneumoniae* (90.7%). The bacterial isolates resistant to meropenem, imipenem, amikacin and gentamicin were 98.64%, 97.94%, 94% and 93%, respectively. Resistance of CRE to all classes of cephalosporin groups, quinolone and combination drugs was 100%. On the other hand, CRE preserved 100% sensitivity to polymyxin-B and colistin. Out of 65 CRE isolates, we detected *bla<sub>OXA-48</sub>* alone in 54%, *bla<sub>NDM</sub>* alone in 22 %, *bla<sub>KPC</sub>* alone in 9.2 % and coexistence of more than one gene in 14.5% of isolates, as 10% (7/65) of the isolates showed (*bla<sub>OXA-48</sub>* + *bla<sub>NDM</sub>*). While, 3% (2/65) have (*bla<sub>OXA-48</sub>* + *bla<sub>KPC</sub>*) and others 1.5% (1/65) have (*bla<sub>NDM</sub>* + *bla<sub>KPC</sub>*). We didn't detect neither IMP nor VIM genes. Multiplex PCR was superior to phenotypic methods in detection and identification of carbapenemase genes. Among phenotypic methods, mCIM was the most sensitive for detection of carbapenemase production.

**Keywords:** Carbapenem-resistance; *Enterobacteriaceae*; API20E system; MALDI-TOF/MS; phenotypic and genotypic detection.

### INTRODUCTION

Gram-negative bacteria represented as a common commensals in nature and cause infection in multiple body sites including the respiratory tract, bloodstream and urinary tract among others[1], even in some cases Gram-negative bacteria causes urogenital diseases, Farrag *et al.*, reported that some parts of the world, oligospermia and azoospermia has been reported due to infection caused by *Enterobacteriaceae* include either cystourethritis, caused by trivial urinary bacteria or by sexually transmitted pathogens affecting fertility [2,3].

Over the last decades, antimicrobial resistance emerged to a wide range of antibiotics [3]. Carbapenems possess broad-spectrum antimicrobial activity and unique structure that provides protection against most  $\beta$  lactamases such extended-spectrum  $\beta$ -lactamases, and hence are considered the most potent antimicrobial against Gram-negative infections [5]. Infection with carbapenem-resistant *Enterobacteriaceae* (CRE) is considered as an urgent challenge in healthcare settings and a growing concern worldwide [5,6]. CRE have caused a broad range of nosocomial and/or community-acquired infections, for instance, urinary tract infections (UTI),

pneumonia, bloodstream infections (BSI), intra-abdominal infections (IAI), skin and soft tissue infections (SSTI), leading to high mortality and morbidity rates [7,8,9].

Resistance to carbapenems among *Enterobacteriaceae* results from multiple mechanisms of resistance, including enzymatic inactivation, target-site mutation, and efflux pumps [5]. The recent emerging of Metallo- $\beta$ -lactamases and other  $\beta$ -lactamases has hugely impacted the utility of carbapenems that considered the last resort antimicrobial agent [10]. A large variety of carbapenemases have been identified in *Enterobacteriaceae* belonging to three classes of  $\beta$ -lactamases: the Ambler classes A, B and D, some these classes are chromosomally encoded and others are plasmid-encoded [11, 12, 13]. Moreover, the plasmid-mediated resistance can spread among different bacterial isolates [5]. Therefore, highly sensitive and specific methods for identification of bacterial isolates and detection of carbapenemases in clinical laboratory settings are in demand [14, 15, 16].

Phenotypic tests can initially predict the presence of carbapenemase production and are considered the easiest and most cost-effective methods, especially in limited laboratory setup, nevertheless they lack specificity regarding the type of carbapenemase being produced [17,18].

Phenotypic assays depend on either growth-based assays such as modified Hodge test (MHT) and modified carbapenem inactivation method (mCIM) that have been proposed in CLSI (2017) guidelines [19,20,21] or hydrolysis methods which detect the product of hydrolysis that is catalyzed by carbapenemase enzymes such as Carba-NP and matrix-assisted laser desorption-ionization-time of flight mass spectrometry (MALDI-TOF MS) methods[22].

Molecular techniques have become an efficient tool for carbapenemase detection and characterization, and are currently considered to be the golden standard method. These are mostly focus on the detection of the carbapenem resistance genes in *Enterobacteriaceae*. More recently, multiplex PCR (Polymerase chain reactions) have been used for the detection of several classes of carbapenemases [5]. In Egypt, data on the mechanisms of carbapenem-resistance among clinical isolates of *Enterobacteriaceae* and methods of their

detection are sparse. Therefore, the aim of this study was to evaluate the sensitivity and specificity of three phenotypic methods, (MHT), (EDTA\CDT) and (mCIM) in comparison with the PCR method in identification of 65 (CRE) isolates from patients admitted to a private tertiary care hospital.

## MATERIALS AND METHODS

### 1- Study design and sample collection

A retrospective study conducted during period from April 2017 to April 2019, on patients hospitalized in the intensive care units (ICU) in private tertiary care hospital, and confirmed positive culture with CRE. All the cases enrolled in the study were cancer patients, hepatitis patients, urinary tract infection patient and blood stream infection patient. The clinical isolates were collected from blood, ascetic fluid, sputum, urine, drain, broncho-alveolar lavage and endotracheal tubes under complete aseptic conditions by physicians before administration of antibiotics. All the samples and samples from positive blood culture bottles also were recovered primary on both blood and MacConkey agar plates (Oxid Ltd. England). Then the plates were incubated at 37°C for 18 to 24 h. Based on growth on MacConkey agar, isolates were identified as lactose fermenters appeared as pink colonies [23].

### 2- Identification of bacterial isolates

All isolates were identified with routine biochemical tests such as Triple sugar iron agar (TSI), Citrate utilization test (CIT) , Urea agar test (UREA), Lysine iron agar (LIA) ,Motility/ Indole/ Ornithine media (MIO) all media mentioned above were obtained from Oxid Ltd. England except MIO which was obtained from Hi media India [24]. Identification was confirmed by The API 20E system for *Enterobacteriaceae* (Bio-Mérieux, Marcy l'Etoile, France) and (MALDI-TOF) mass spectrometry [25]

### 3- Antibiotic susceptibility testing

Susceptibility testing was done using a modified Kirby Bauer disc diffusion method on Muller Hinton agar (Oxoid Ltd) [26], and according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [21]. A total of 15 types of antibiotic discs, were classified into 10 antimicrobial categories which

were used: (Oxoid Ltd) penicillins (ampicillin 10 µg), one of the β-lactam/β-lactamase inhibitors (amoxicillin /clavulanic acid 30 µg), monobactam (Aztreonam 30 µg), antipseudomonal penicillins+ β-lactamase inhibitor (piperacillin/ tazobactam 110 µg), three of extended spectrum cephalosporins; 3<sup>rd</sup> generations (ceftazidime, 30 µg; cefotaxime, 30 µg; ceftriaxone, 30 µg), Extended spectrum cephalosporins; 4<sup>th</sup> generations (cefepime 30 µg), aminoglycosides (amikacin, 30 µg; gentamicin, 120 µg), fluoroquinolones (ciprofloxacin 5 µg), carbapenems (imipenem, 10 µg; meropenem, 10 µg), and cyclic polypeptide (tigecycline and polymyxin B), after overnight incubation at 37°C. Isolates of *Enterobacteriaceae* which were resistant to either imipenem or meropenem or both, were selected for further phenotypic and molecular testing. *Escherichia coli* ATCC 25922 were used as a control strain.

#### 4- Phenotypic detection of carbapenemase activity

Isolates that showed reduced susceptibility to carbapenem considered for further investigation of carbapenemase production by Modified Hodge test (MHT) that was performed according to CLSI guidelines [27], as the clover leaf-like appearance between the test streaks near the disc was taken as positive for carbapenemase production. While the detection of MBLs (class B) was performed using a combined disk test of imipenem with EDTA (CDT-IMP+EDTA). A zone diameter difference

of >7 mm between imipenem disks and imipenem plus EDTA was interpreted as MBL-positive [28]. and detection carbapenemase hydrolysis was performed using modified carbapenem inactivation method (mCIM) which determine either the meropenem had been hydrolyzed by carbapenemase activity (growth of an indicator organism close to the disk), or the meropenem is still active (appearance of large inhibition zone around the disk) [21].

#### 5- Genotypic analysis for carbapenemase encoded genes by Polymerase Chain Reaction (PCR)

*bla<sub>VIM</sub>*, *bla<sub>IMP</sub>*, *bla<sub>KPC</sub>*, *bla<sub>NDM</sub>* and *bla<sub>OXA-48</sub>* genes were detected and amplified by a multiplex PCR assay according to (Poirel *et al*). The design of the primers used for the detection of are given in Table (1) [29].

#### 6- Statistical Analysis

IBM SPSS statistics (V. 26.0, IBM Corp., USA, 2019) was used for data analysis. Data were expressed as both number and percentage for categorized data.

The following tests were done:

1. Chi-square test to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data.  
The probability of error at 0.05 was considered sig., while at 0.01 and 0.001 are highly sig.
2. Diagnostic validity test: It includes percent agreement between 2 items.

**Table (1): Primer sequence used for carbapenemase encoded genes and their size.**

Target gene	Sequence (5' – 3')	Expected PCR amplicon size (bp)	Reference
<i>bla<sub>OXA-48</sub></i>	F: 5' GCGTGGTTAAGGATGAACAC 3' R: 5' CATCAAGTTCAACCCAACCG 3'	438	Poirel <i>et al</i> ,
<i>bla<sub>KPC</sub></i>	F: 5' CGTCTAGTTCTGCTGTCTTG 3' R: 5' CTGTGCATCCTTGTTAGGCG 3'	798	
<i>bla<sub>NDM</sub></i>	F: 5' GGGTTGGCGATCTGGTTTTTC 3' R: 5' CGGAATGGCTCATCACGATC3'	621	
<i>bla<sub>VIM</sub></i>	F: 5' GTTTGGTTCGCATATCGCAAC 3' R: 5' AATGCGCAGCACCAGGATAG 3'	382	
<i>bla<sub>IMP</sub></i>	F: 5'GCATAAGTCGCAATCCCCG 3' R: 5'CTTCTATCTCGACATGCCG 3'	237	

## RESULTS

Out of 250 non duplicate enterobacterial isolates recovered from different clinical specimens during the study period 26 % (65/250) were CRE. The types of specimens show the highest rate of CRE isolates was isolated from blood cultures 35.4% (23/65), followed by from endotracheal tube 21.5%(14/65), from sputum 20% (13/65), from urine 9% (6/65), and from wound swab 7.7%(5/65), from drain 3% (2/65), from ascetic fluid 1.5% (1/65) and from BAL 1.5% (1/65) as shown in Table (2).

**Table (2): Distribution of the source specimens for CRE according to clinical samples**

			Total	Probability of error (P)
Specimen	Ascetic Fluid	Count	1	P-Value 0.038
		%	1.5%	
	BAL	Count	1	
		%	1.5%	
	Blood	Count	23	
		%	35.4%	
	Drain	Count	2	
		%	3.1%	
	E.T.T	Count	14	
		%	21.5%	
	Sputum	Count	13	
		%	20.0%	
	Urine	Count	6	
		%	9.2%	
	W.S	Count	5	
		%	7.7%	
Total		Count	65	
		%	100.0%	

\* ETT, Endotracheal tube; W.S,Wound Swab.

### 1. Identification of bacterial isolates

The most common species were *K. pneumoniae* represented as 90.8% (59/65) followed by *E. cloacae* 4.6 (3/65), *E.coli* 3% (2/65) and *P. vulgaris* are 1.5(1 /65) Table (3).

**Table (3): The frequencies of isolated CRE species according to its species.**

organisms	Total	Probability of error (P)
<i>E. cloacae</i>	Count	3
	%	4.6%
<i>E.coli</i>	Count	2
	%	3.1%
<i>K. pneumoniae</i>	Count	59
	%	90.8%
<i>P. vulgaris</i>	Count	1
	%	1.5%
Total	Count	65
	%	100.0%

### 2. Antibiotic susceptibility testing by disc diffusion method:

The bacterial isolates resistant to meropenem and imepenem were 98.64% (64/65) and 97.94% (63/65), respectively. Resistance to amikacin and gentamicin was 94% (61/65) and 93% (60/65) respectively. Resistance of CRE to all classes of cephalosporin groups, quinolone and combination drugs was 100%. On the other hand, CRE preserved 100% sensitivity polymyxin-B and colistin.

### 3. Phenotypic Detection of carbapenemase

#### 3.1. Detection by the Modified Hodge test (MHT).

Out of the 65 CRE isolates, 63.10% (41/65) were positive for MHT, of which *K. pneumoniae* was the most common carbapenemase producer as it constituted 56.9 % (37/65) of the total resistant CRE isolates, followed by *E. cloacae* that constituted 4.6% (3/65), *E. coli* constituted 1.5% (1/65) and *P. vulgaris* were Negative for MHT.

#### 3.2. Detection of potential Carbapenemases by EDTA-CDT

Phenotypic detection of MBL was done by EDTA-CDT. Among the 65 isolates of CRE, 38.46% (25/65) were positive EDTA-CDT. Of which 33.8% (22/65) were *K. pneumoniae*, followed by *E. coli* at 3.0% (2/65) and *E. cloacae* at 1.5 % (1/65), while *P. vulgaris* were negative for EDTA-CDT.

### 3.3. Detection by the modified carbapenem inactivation Method (mCIM)

Our results show that 100 % (65/65) of isolated CRE were positive mCIM. However, some CRE isolates gave positive results with more than one phenotypic method. 13.8% (9/65) of CRE isolates gave positive result with (MHT+MBL+mCIM). Of which 10.7 % (7/65) were *K. pneumoniae* , 1.5%(1/65) *E. cloacae* and 1.5%(1/65) were *E.coli* while 49.2% (32/65) of CRE isolates give positive result with (MHT + mCIM) of which 46.1% (30/65) were *K. pneumoniae* and 3.0%(2/65) were *E. cloacae* ,and 24.6% (16/65) of CRE isolates give positive result with (MBL+mCIM) of which 23.1% (15/65) were *K. pneumoniae*, and 1.5% (1/65) were *E.coli*. While the overall description of phenotypic methods used for detection of isolated CRE were shown in Figure (1).

### 4. Genotypic detection of resistance genes

Multiplex PCR was used for detection of most commonly carbapenemase encoding genes

*bla<sub>KPC</sub>* for class A carbapenemase, (*bla<sub>NDM</sub>*, *bla<sub>VIM</sub>*, *bla<sub>IMP</sub>*) for class B carbapenemases, and *bla<sub>OXA-48-Like</sub>* for class D carbapenemases. All the 65 isolates showed presence of at least one of carbapenemase encoded genes by the multiplex PCR assay as showed in Figure (2).

As shown in Table (4), *bla<sub>OXA-48-Like</sub>* gene was detected in 67.6% (44/65) of isolated CRE. *K.pneumoniae* were the major organism that harbored the *bla<sub>OXA-48-Like</sub>* gene, which constituted 60% (39/65) followed by *E. cloacae* 4.6% (3/65), *E.coli* 1.5 % (1/65), and *P. vulgaris* 1.5% (1/65). On the other hand, *bla<sub>NDM</sub>* gene was detected in 33.8% (22/65) of isolated CRE, *K. pneumoniae* showed 29.2 % (19/65) followed by *E. coli* at 3.08 % (2/65), *E. cloacae* at 1.54%(1/65), while *P. vulgaris* of isolated CRE were negative for *bla<sub>NDM</sub>* genes. Besides this, *bla<sub>KPC</sub>* gene was detected in 13.8% (9/65) of isolated CRE; all of them were *K. pneumoniae*.

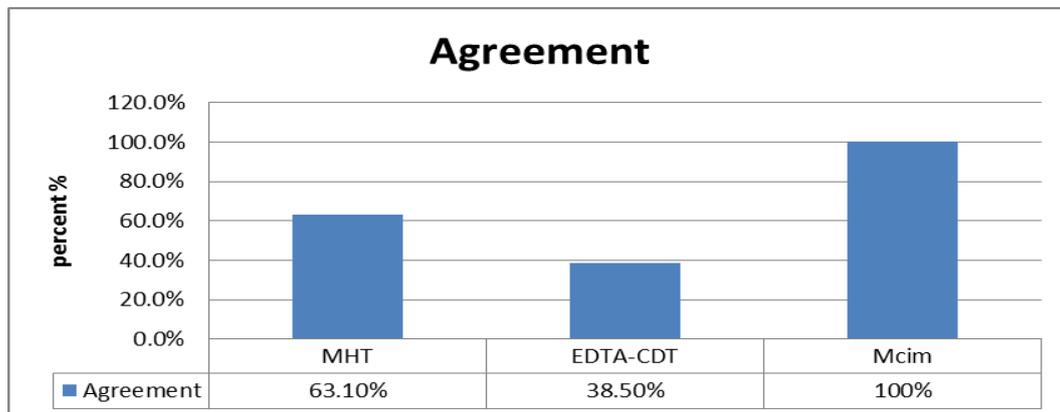


Figure (1): Percentage of phenotypic methods Agreement.

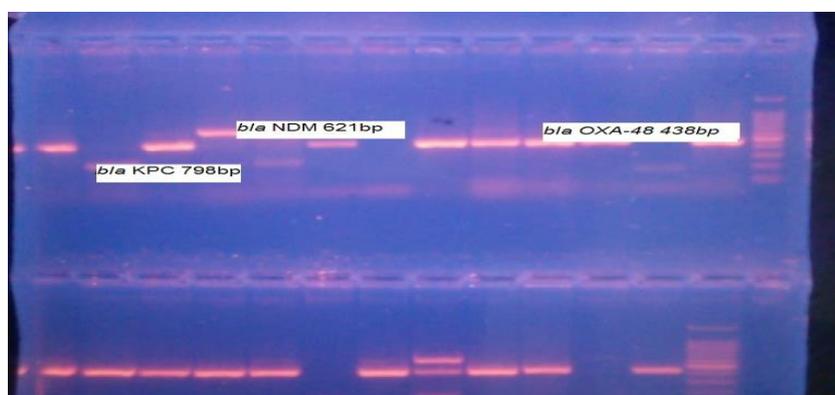


Figure (2): Multiplex PCR for detection of *bla<sub>OXA-48</sub>*, *bla<sub>NDM</sub>*, and *bla<sub>KPC</sub>* positive.

**Table (4): Frequencies of carbapenemase gene among isolated CRE.**

Bacterial isolate	% <i>bla</i> <sub>OXA48</sub>	% <i>bla</i> <sub>NDM</sub>	% <i>bla</i> <sub>KPC</sub>
<i>K. pneumoniae</i>	60	29.23	13.85
<i>E. cloacae.</i>	4.62	1.54	0
<i>E. coli</i>	1.5	3.08	0
<i>P. vulgaris</i>	1.5	0	0
<b>Total</b>	67.69	33.85	13.85

Out of 65 CRE strains included in the study some CRE isolates contain only one resistant gene as *bla*<sub>OXA-48</sub> alone 54% (35/65), *bla*<sub>NDM</sub> alone 22 % (14/65) and *bla*<sub>KPC</sub> alone 9.2 % (6/65). However, CRE showed coexistence of more than one carbapenemase gene as 10% (7/65) of the isolates showed (*bla*<sub>OXA-48</sub> + *bla*<sub>NDM</sub>), while 3% (2/65) have (*bla*<sub>OXA-48</sub> + *bla*<sub>KPC</sub>) and others 1.5 % (1/65) have (*bla*<sub>NDM</sub> + *bla*<sub>KPC</sub>).

### 5. Evaluation of phenotypic methods for detection of carbapenemase genes

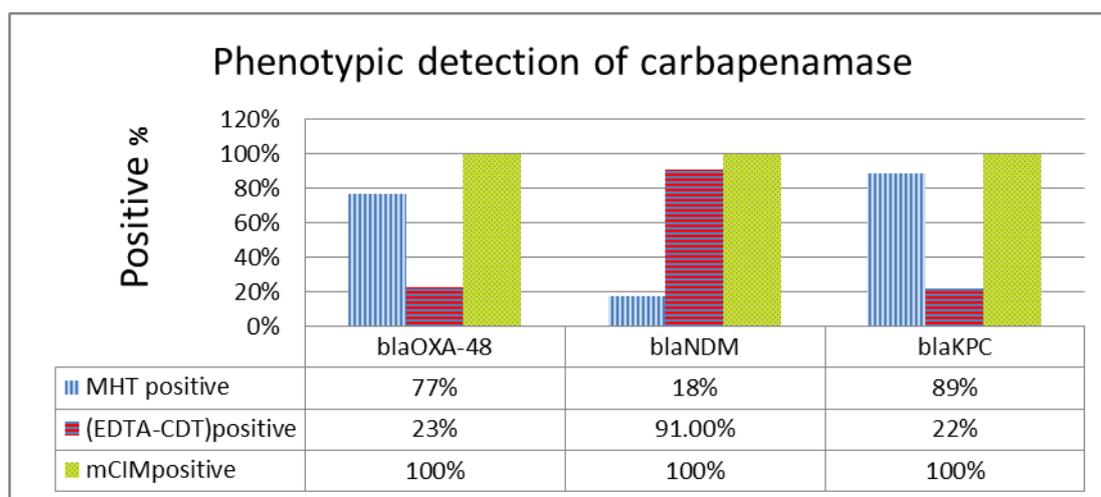
The results showed that the MHT method is a good detector of *bla*<sub>OXA-48</sub> and *bla*<sub>KPC</sub> producing organisms. Of 44 CRE isolates producing *bla*<sub>OXA-48</sub>, 77% (34/44) were detected by MHT. While from 9 cases of *bla*<sub>KPC</sub> producing isolates MHT detected 89% (8/9) of it. Also MHT detected only 18 (4/22) of CRE

containing *bla*<sub>NDM</sub> genes. EDTA-CDT showed a notable detection rate of *bla*<sub>NDM</sub> producing CRE isolates, out of 22 *bla*<sub>NDM</sub> CRE producers EDTA-CDT detected 91% (20/22). While EDTA-CDT detecting 23% (10/44) of CRE containing *bla*<sub>OXA-48</sub> and detected 22.2% (2/9) of CRE containing *bla*<sub>KPC</sub>. On the other hand depending on mCIM for detection of carbapenemase, it was observed that mCIM detected carbapenemase in all isolated CRE which matching the results of the gold standard PCR method (100%), as shown in (figure 3).

### DISCUSSIONS

Infections caused by CRE are of particular concern [30]. These organisms are highly efficient at acquiring genes that code for mechanisms of antibiotic resistance, especially in the presence of antibiotic selection pressure. Furthermore, they contain a variety of resistance pathways, and often contain multiple mechanisms targeting the same antibiotic.

In the present study, 35.5% (23/65) of CRE was isolated from blood specimens that represented the highest rate followed by endotracheal secretion 21.5% (14 /65); Sputum 20% (13/65) and urine 9.32% (6/65). Our findings are in accordance with **Sood *et al***, in which 25% (15/60) of the CER isolates were isolated from blood specimens, followed by endotracheal secretions 18.33% (11/60), sputum



**Figure (3): Relation of phenotypic methods to detect carbapenemase activity in relation to the type of carbapenemase genes.**

11.66% (7/47) and 5% (3/47) from urine [31]. On the other hand, **Pawar et al.**, documented that urine samples were the leading specimen which contributed 48.2% (27/56) of CRE [17]. It is noteworthy to mention that many of the patients were immune compromised, hemato/oncology or bone marrow transplantation patients which related to utilization of intravascular devices and previous administration of antimicrobial agents.

In the present study carbapenem resistant *K. pneumoniae* represented 90.8% (59/65), followed by *E. cloacae*, *E. coli* and *P. vulgaris* at 4.6% (3/65), 3.1% (2/65), and 1.5 (1/65), respectively. Egyptian recent study done by **Abdualall et al.**, revealed that *K. pneumoniae* is the most common pathogen causing catheter related blood stream infection (47.4%), followed by *E. coli*, *E. cloacae*, *A. baumannii*, and *P. aeruginosa* [32]. This is in keeping with recent reports that multidrug-resistant *K. pneumoniae* strains were a common colonizer in the hospital settings and main bloodstream infection pathogens in immunocompromised patients [33].

As regards susceptibility testing; 100% of the isolates showed sensitivity to colistin and polymyxin-B which is consistent with the results of study reported from India by **Sood et al.** that reported 100% (60/60) sensitivities to colistin and polymyxin-B [31]. On the other hand, the present study showed relatively high resistant rates among amikacin and gentamicin 7.6% and 6.1% respectively; in addition, 100% of the isolates showed resistance to ciprofloxacin, cefotaxime, ceftazidime, cefotrixone, ampicillin, augmentin, piperacillin/tazobactam, Aztreonam, and cefepime. Obviously, the present study showed reduced susceptibility to commonly used antibiotics in the hospital settings due to lack of national antimicrobial stewardship program, misuse and overuse of antibiotics unrestricted empirical antimicrobial therapy and the inconsistency of implementation of national infection control guidelines [32,34].

Phenotypic tests, like the MHT is widely used in clinical laboratories as first-line phenotypic methods for detection of the carbapenemase-producing isolates [35]. The results of carbapenemase screening tests by MHT showed that 63.07% (41/65) of CRE isolates were positive; these result is relatively

consistent with the recent study of **Qadri et al.**, who reported that out of the total 52 CRE isolates, 67.3% (35/52) were MHT positive [36].

In the present study, 38.4% (25/65) showed positive result to EDTA-CDT. As well as, 91% (20/22) of *bla<sub>NDM</sub>* producers showed positive results for EDTA-CDT. These results were in agreement with **Pawar et al.**, who reported that 89 % (59/66) of isolates were positive for EDTA-CDT and 82 % (54/66) showed presence of *bla<sub>NDM-1</sub>* [17]. However, EDTA-CDT couldn't detect 78% (7/9) of CRE that containing *bla<sub>KPC</sub>* and 77% (34/44) of *bla<sub>OXA-48</sub>* harboring. Accordingly, **Chu et al.**, reported that MBL inhibitor (EDTA) may possess their own bactericidal activity, which may result in expanded inhibition zones not associated with true MBL production and hence false positive results occur. Also the false positive results may be due to increase outer membrane permeability caused by EDTA used in the test [9,37]. On the other hand, **Picao et al.**, reported that false-negative results might arise from carbapenem hydrolysis or inactivation caused by EDTA [38]. **Franklin et al.**, showed that *bla<sub>NDM</sub>* gene is carried on plasmids which also carry a number of other genes conferring resistance to aminoglycosides, macrolides and sulphamethoxazole, thus making these isolates multidrug resistant [39].

In the present study the mCIM that is a new phenotypic method for detection of carbapenemases activity in CRE was positive in all 65 CP-CRE strains which is completely consistent to the result of **Foldes et al.**, where the mCIM was positive in all 19 CP-CRE strains and no false-positive results were noticed [40]. This is also relatively consistent to **Pawar et al.**, who reported that only one isolate which was positive by PCR study was negative by mCIM method [17], making this new method most sensitive (98.48%) among the phenotypic test studied. **Pawar et al.**, shows that the only disadvantages of mCIM are its inability to discriminate the type of carbapenemase and it is time consuming [17].

However, the phenotypic tests have their own limitations, being time-consuming, difficult to interpret and unable to accurately differentiate between carbapenemases responsible for carbapenem resistance. This necessitated further testing by genotypic

methods which can determine the most prevalent carbapenemase genes including five common and predominant carbapenemase genes (*bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>OXA-48-like</sub>, *bla*<sub>VIM</sub>, and *bla*<sub>IMP</sub>) in less than an hour.

Our study highlights that the most prevalent carbapenemase gene was *bla*<sub>OXA-48-like</sub> at 67.7 % (44/65), while *bla*<sub>NDM</sub> represented 33.8 % (22/65), *bla*<sub>KPC</sub> 14 % (9/65), *bla*<sub>IMP</sub> and *bla*<sub>VIM</sub> genes were not detected. This is in keeping with a recent study by **El-Kholy *et al.***, who reported that *bla*<sub>OXA-48-like</sub> dominated (40.6%), followed by *bla*<sub>NDM-1</sub> (23.7%) and *bla*<sub>OXA-232</sub> (4.5%) [41]. In contrast, **Abdulall *et al.***, showed that *bla*<sub>NDM</sub> as the predominant gene in 48.1% of *K. pneumoniae*, and in 14.3% of *A. baumannii* [32]. This is in agreement with previous studies that reported the predominance of *bla*<sub>NDM</sub> in Egypt and Middle East [33, 42, 43].

Our results showed the limited sensitivity of MHT in detection of carbapenem production, as it detected only 18% (4/22) for detection of *bla*<sub>NDM</sub> genes positive, 77% of *bla*<sub>OXA-48-Like</sub> positive and 89% of *bla*<sub>KPC</sub> positive isolates. These results are in broad agreement with **Doyle *et al.***, results that MHT had the sensitivity of 98 % for detection of *bla*<sub>KPC</sub> harboring, 93 % for *bla*<sub>OXA-48-like</sub> [44]. Apart from being time consuming, disadvantages of this test (MHT) include interpretation difficulties and the inability to distinguish between different classes of carbapenemases [45,46]. In recent Clinical Laboratory Standard Institute guidelines CLSI (2019), MHT is no longer included as a reliable phenotypic method for carbapenemase detection and other methods such as the CarbaNP test and the mCIM are more reliable [47]. CRE showed coexistence of more than one carbapenemase gene. This was in agreement with other Egyptian results [31,41,42]. Although, the distribution of the carbapenemase genes varied in different Egyptian studies, yet both NDM and OXA-48 genes were the most predominant.

## CONCLUSION

Carbapenem resistance in Egypt is increasing, and OXA-48 is the most common source of carbapenem resistance in *Enterobacteriaceae*. Elderly population and ICU admission were important risk factors for CRE acquisition. Accurate detection of carbapenemase producing genes by molecular methods overcomes the problem related to CRE.

Though there is no signal method that is ideal for all situations, the mCIM method is simple, less subjective, cost effective, reproducible and most sensitive method for detection of CRE.

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**الملخص العربي:****تقييم الفروق بين الطرق المظهرية والطرق الجينية المستخدمة للكشف عن البكتريا المعوية الممرضة المقاومة للكاربابينيم.**

يعتبر ظهور البكتريا المعوية المضادة للكاربابينيم في السنوات الأخيرة مشكلة خطيرة ومزعجة حيث تنزايد طرق المقاومة المتعددة للعقاقير الطبية بشكل كبير في البكتريا المعوية المسببة للعدوى المكتسبة من المستشفيات والمجتمع في الوقت الحالي. و الكاربابينيم هو فئة من المضادات الحيوية المستخدمة لمعالجة التهابات الشديدة؛ وأيضاً في معالجة الحالات التي تفشل في علاجها معظم المضادات الحيوية الأخرى شائعة الاستخدام حالياً. في بعض الحالات تكون البكتيريا المقاومة لمضادات كاربابينيم مقاومة أيضاً لجميع المضادات الحيوية المتاحة وهذا يكون بسبب ان الجينات الخاصة بانتاج إنزيمات الكاربابينيميز بواسطة البكتريا المعوية تكون محملة على بلازميد وهو جزء من الحمض النووي يستطيع الانتقال بسهولة من بكتيريا إلى أخرى. وهكذا انتشرت المقاومة ضد عائلة الكاربابينيم بين البكتريا المعوية ولان البلازميد يحتوي أيضاً على جينات خاصة بمقاومة باقي المضادات الحيوية والتي تعطى مقاومة تقريباً لكل البيتا لكتام كذلك يمكن للبكتيريا المعوية المقاومة لكاربابينيم ان تنتقل من شخص إلى آخر خاصة مرضى نقص المناعة مثل مرضى السكر والاطفال والحوامل وكبار السن تعتبر البكتريا المعوية المضادة للكاربابينيم مسؤولة عن حالات تفشي الأمراض في أماكن الرعاية الصحية خاصة الأشخاص الذين تتطلب رعايتهم أجهزة مثل أجهزة التنفس أو القسطرات وكذلك المرضى المحجوزين في وحدات الرعاية المركزة لفترة طويلة وتعتبر كذلك السبب في تزايد نسبة الوفيات. يمكن للبكتيريا المقاومة للمضادات الحيوية أن تسبب التهابات في الرئتين (الالتهاب الرئوي) والمسالك البولية والجلد وامراض الدم. لذلك هذه الدراسة تهدف الى تقييم الفروق بين الطرق المظهرية والطرق الجينية المستخدمة في المستشفيات والمعامل والمراكز الصحية للكشف عن البكتريا المعوية المقاومة لمضادات كاربابينيم الحيوية. وقد اكدت نتائج الدراسة ان بكتيريا كليبسيلا نيومونيا هي من اكثر البكتريا المعوية المضادة للكاربابينيم انتشاراً يليها الإشريشية كولاى ومن اكثر إنزيمات الكاربابينيميز انتشاراً نيودلهي ميتالو بيتا لاكتاميز و كليبسيلا كاربابينيميز والاكسا- 48 . لذلك يجب أن تكون المعامل والمختبرات قادرة على الكشف وتحديد هذه الإنزيمات بين الجراثيم المعوية بسرعة لمنع تطور ذلك الوباء في المستشفيات. وتستغرق الطرق التقليدية الحالية القائمة على زراعة البكتريا 24 ساعة لتحديدها مما يتسبب في تأخير العلاج بالمضادات الحيوية الفعالة والسماح لانتقال البكتريا المنتجة للكاربابينيم التي لم يتم الكشف عنها بين البكتريا المعوية الأخرى. تم اجراء الدراسة الحلية على 65 سلالة من البكتريا المعوية المقاومة للكاربابينيم والعزولة من مرضى الرعاية المركزة في مستشفى خاص في الفترة من ابريل 2017 الى ابريل 2019. تم جمع العينات تحت ظروف معقمة جداً بواسطة الطبيب المعالج وتم تجميعها من مصادر مختلفة تشمل: الدم , البصاق , البول , مسحات من الجروح وكذلك سائل الاستصقاء. وقد اظهرت الدراسة ان عدوى الدم كانت من اهم واعلى نسب احتواء البكتريا المعوية المقومة للكاربابينيم والتي تمثل 35%(65/23) تليها عدوى الجهاز التنفسي مثل عينات (البصاق والانبوبة الحنجرية) والتي تمثل (21% و 20%) على التوالي ثم عدوى الجهاز البولي والتي تمثل 9% . خضعت جميع العزلات للتعريف عن طريق عمل العديد من الاختبارات البيوكيميائية مثل : تخمر السكريات و انتاج انزيمات يوريباز، وقدرتها على الإستفادة من السترات، أورنيسين ديكاربوكسيليز، و ليسين ديكاربوكسيليز، و كذلك اختبار قدرة البكتريا على الحركة و انتاج الإندول. كذلك خضعت جميع العزلات التي تم تعريفها بالاختبارات السابقة للتعرف الدقيق باستخدام (MALDI- TOF) MS, API 20E system) وقد اكدت نتائج الدراسة ان بكتيريا كليبسيلا نيومونيا هي من اكثر البكتريا المعوية المضادة للكاربابينيم انتشاراً يليها الأنثيروبكتير كلواكي و الإشريشية كولاى ثم البروتيس فولجاريس بنسب (90.7%, 4.6%, 3% و 1.6%) على التوالي. تم عمل اختبارات حساسية للسلاطات المعزولة لعدد 15 نوع من المضادات الحيوية والتي تمثل 10 مجموعات من المضادات الحيوية شائعة الاستخدام خاصة في المستشفيات. وقد اظهرت نتائج الحساسية مقاومة هذه العزلات البكتيرية لمضادات الكاربابينيم (ميروبيينم واميبينيم) بنسب (89.6% و 97.9%) على التوالي. كذلك مقاومتها لمجموعة الامينوجليكوزيد (اميكاسين وجينتاميسين) بنسب (94% و 93%) على التوالي وكذلك مقومة بنسبة 100% لباقي المضادات الحيوية المستخدمة . كذلك اظهرت النتائج حساسية بنسبة 100% لمضادات البولي ميكسين- بي والكوليسيتين. ولقد دفعنا مقاومة هذه العزلات للمضادات الحيوية وخاصة مقاومتها لمجموعة الكاربابينيم الى دراسة اليات هذه المقاومة والطرق المظهرية وكذلك الطرق الجينية المستخدمة للتعرف السريع والدقيق على هذه السلالات المقاومة للمضادات الحيوية. ومن الطرق المظهرية المستخدمة.