Original article

Performance of chromID® CARBA-SMART medium and carbapenemase inhibition method for the detection of carbapenemases among Gram negative bacilli

Aliaa M.A. Ghandour *1, Wegdan A. Mohamad 1, Rania M. Bakry 2, Asmaa Omar Ahmed 3, Nahla M. Elsherbiny 1

1- Medical Microbiology and Immunology Department, Faculty of Medicine, Assiut University, Assiut, Egypt.
2- Department of Oncological Clinical Pathology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt.
3- Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.

A R T I C L E   I N F O

A B S T R A C T

Background: Early detection of carbapenemase enzymes among Gram negative bacilli (GNB) is mandatory to prevent their spread. **Objective:** The goal of this study was to evaluate the performance of chromID® CARBA-SMART medium and carbapenemase inhibition method (CIM) for detection of carbapenemases in GNB. **Methodology:** A total of 142 GNB isolates were collected and tested using Vitek-2® system for identification and antimicrobial susceptibility testing The carbapenem resistant (CR) GNB were tested for carbapenemase production by phenotypic methods; chromID® CARBA-SMART medium and CIM. Carbapenemase genes (bla_{NDM-1}, bla_{SIM-1}, bla_{VIM-2}, bla_{KPC-1}, bla_{GIM-1}, bla_{SPM-1} and bla_{OXA-48}) were detected by PCR. **Results:** By minimal inhibitory concentration (MIC), 111 (78.17 %) of the 142 isolates were shown to be carbapenem resistant. Sensitivity and specificity of CIM and ChromID® CARBA-SMART medium were (100% and 66.7% respectively) for CIM and (86.7% and 100% respectively) for the medium. Resistance to carbapenem was associated with high percentages of resistance to many antibiotic classes. Carbapenemase genes were detected in 82% (91/111) of CR GNB with bla_{NDM-1} (58.6%) and bla_{OXA-48} (55.9 %) having the highest prevalence, followed by bla_{KPC-1} (36 %) then bla_{VIM-2} (10.8%) and lastly bla_{SPM-1} (3.6 %) and bla_{SIM-1} (1.8 %). The $blaGIM_{-1}$ gene was not detected in any isolate. **Conclusion:** Carbapenemase inhibition method was found to be a very sensitive, easy, and cheap test for carbapenemase detection but needs the addition of ChromID® CARBA-SMART medium to improve the specificity of the test. This study has a high prevalence of carbapenemases among isolates with potential of rapid spread necessitating need for phenotypic tests.

Introduction

Carbapenems (ertapenem, meropenem, doripenem and imipenem) are the preferred antibiotics for life-threatening infections caused by multidrug resistant *Enterobacteriaceae* as they have the widest spectrum of antibacterial activities and are active against the extended spectrum beta lactamases and chromosomal cephalosporinases [1].

Carbapenem resistance can be conferred by many mechanisms, one of which is the production of carbapenem hydrolyzing enzymes

* *Corresponding author:* Aliaa M.A. Ghandour
E-mail address: aliaaghandour@aun.edu.eg

(carbapenemases), which is the most concerning mechanism of carbapenem resistance [2].

The Ambler classification, which is based on sequence and structural homology, was used to classify acquired carbapenem hydrolyzing enzymes [3]. Carbapenemases are classified into two groups based on the content of active sites: (i) serine carbapenemases classified into the class A penicillinases such as KPC (*Klebsiella pneumoniae* carbapenemase), GES, SME, NMC and IMI categories and class D oxacillinases like OXA-48 and its variations, which include a serine at the active site and become inactive by clavulanic acid and tazobactam as β-lactamase inhibitors. (ii) metallo-β-lactamases classified into the class B carbapenemases like NDM (New Delhi metallo- β - lactamases), VIM (Verona integron encoded metallo- β -lactamase), IMP, GIM, SIM and SPM types, which include zinc atoms at the active site, hydrolyzing β-lactam ring. These enzymes are inactivated by EDTA [3,4].

These carbapenemase genes are usually found on determinants encoded by transposons and/or integrons. In contrast to noncarpapenemase-related mechanisms, which are nontransferable, they have the potential for extensive transmission and consequently the spread of carbapenem resistance [4].

Therefore, the accurate identification of such enzyme producers is of utmost importance for the proper containment and management for effective infection control policies [5].

In Egypt, most hospitals have limited resources to confirm the presence of carbapenemase production in clinical isolates. Thus, the need for a laboratory test which is accurate, cost effective and easy to perform is highly recommended. Genotypic methods, though highly sensitive and specific, can't be performed routinely as they need expensive equipment, trained microbiologists and are time consuming [5].

The chromID CARBA-SMART and the carbapenem inactivation method (CIM) are two quick phenotypic detection procedures for carbapenemases. The chromID CARBA-SMART is used as a chromogenic media for carbapenemase producing *Enterobacteriaceae* detection. It's a bi-plate with "OXA" agar on one side and the original chromID CARB agar on the other [6,7]. The CIM, on the other hand, is based on the ability of a carbapenem susceptible bacteria to grow close to a carbapenem containing disc after incubation of this disc with the suspected carbapenemase producing bacteria [8].

This study aimed to evaluate performance of chromID® CARBA medium and CIM for detecting carbapenemases in Gram negative bacilli (GNB).

Materials and Methods

Ethical statement

With IRB local approval number:17300624 dated 30/6/2021, the Ethical Committee of the Faculty of Medicine at Assiut University, Egypt, accepted the research in conformity with the World Medical Association's code of ethics (Declaration of Helsinki).

Study design

This cross-sectional study was conducted in the Department of Medical Microbiology and Immunology, Assiut University. The bacteria were isolated from clinical samples of patients with hospital acquired infections who were admitted to different intensive care units of Assiut University Hospitals. The samples were analyzed using the Vitek-2® system of South Egypt Cancer Institute, Assiut University, Egypt.

Sample size and bacterial isolates

A total of 142 GNB isolates were collected from various clinical specimens of patients with hospital acquired infections. Isolates were identified using both traditional bacteriological investigations and the Vitek-2® system with GN-ID cards (bioMerieux, France) [9]. Luria-Bertani broth (LB liquid medium) (HiMedia, India) and 30% glycerol were employed to preserve bacterial isolates, then to be frozen at -20°C until needed.

Antimicrobial susceptibility test of carbapenemase producing Gram negative bacilli

Susceptibility testing were performed on the isolates using the Vitek-2® system and the AST-GN204 cards (bioMerieux, France) [10].

The isolates were tested for the following antimicrobials: Ampicillin, Amoxicillin / clavulanic acid (β-lactams); piperacillin/tazobactam (β-lactamase-inhibitor combination); cefotaxime, ceftazidime, cefepime and cefoxitin (cephalosporins); gentamicin and amikacin (aminoglycosides); imipenem, ertapenem and meropenem (carbapenems); ciprofloxacin and norfloxacin (quinolone); trimethoprim/sulphamethoxazole (sulphonamide); Fosfomycin and nitrofurantoin.

Phenotypic detection of carbapenemases

The Carbapenem Inactivation Method (CIM)

The CIM was performed as following; A suspension was prepared by mixing inoculation loopful culture (10 μl) from blood or Mueller Hinton agar (MHA) plate with 400 μl water. A disk of 10 μg meropenem (Oxoid, United Kingdom) was dipped in the suspension and incubated at 35°C for two hours. After the 2 hrs incubation, the disk was picked up from the suspension, then placed on MHA plate inoculated with a susceptible *E. coli* indicator strain (ATCC 29522) then incubated at 35°C. If the bacterial isolate was a carbapenemase producer, the meropenem was inactivated giving indicator strain growth. If the bacterial isolate was a non carbapenemase producer, the meropenem inhibited the indicator strain growth [11].

Culture on chromID® CARBA-SMART medium

The ChromID® CARBA-SMART medium (bioMérieux, France) comprises of two chromogenic culture media (CARB/OXA-48) poured into a single Petri plate with distinct compartments. This medium consists of a nutrient base containing combination of three chromogenic substrates that allow the detection of specific metabolic enzyme activities in *E coli*, KESC group (*Klebsiella*/*Enterobacter*/*Serratia*/*Citrobacter*) and *Proteae* (*Proteus*, *Providencia*, *Morganella*), various peptones and a mixture of antibiotics that promote the selective growth of carbapenemase producing *Enterobacteriaceae*, mostly KPC and NDM-1.OXA-48 type carbapenemase producing GNB (CR GNB) grew on the OXA medium and the other CR GNB grew on the CARB medium [7]. Suspected CR GNB colonies were those with pink to burgundy for *E. coli*, purple or blue/green to blue/grey for KESC group, and brown for *Proteeae* [12].

Polymerase chain reaction (PCR) based detection of carbapenemase encoding genes

Primers (Invitrogen Company, UK) targeting bla_{VIM-2}, bla_{KPC-1}, bla_{OXA-48}, bla_{NDM-1}, bla_{SPM-1}, bla_{GIM-1}, and bla_{SIM-1} were used to identify the presence of carbapenemase genes. To extract DNA, cells were lysed using the boiling procedure. The PCR conditions were followed exactly as mentioned before by **Yigit et al.** [13] for bla$_{KPC-1}$, **Gutiérrez et al.** [14] for bla$_{VIM-2}$, **Nordmann et al.** [15] for bla$_{NDM-1}$, **Poirel et al.** [16] for bla_{OXA-48} and **Azim et al.** [17] for bla$_{SPM-1}$, bla$_{GIM-1}$, bla$_{SIM-1}$. The sizes of the amplified products are shown in **table (1)**.

Table 1. Primers used for the detection of carbapenemase encoding genes.

Gene	Primer sequence ('3-'5)	Product size (bp)
bla_{NDM-1}	5′-GGT TTG GCG ATC TGG TTT TC-3′ 5′-CGG AAT GGC TCA TCA CGA TC-3′	621
bla_{SIM-1}	5′-TACAAGGGATTCGGCATCG-3′ 5′-TAATGGCCTGTTCCCATGTG-3′	570
bla_{VIM-2}	5′- ATG TTC AAA CTT TTG AGT AAG-3′ 5′- CTA CTC AAC GAC TGA GCG-3′	801
bla_{KPC-1}	5′- ATG TCA CTG TAT CGC CGT CT -3′ 5′- TTT TCA GAG CCT TAC TGC CC -3′	893
bla_{GIM-1}	5′--TCG ACA CAC CTT GGT CTG AA-3′ 5′-AAC TTC CAA CTT TGC CAT GC-3′	477
bla_{SPM-1}	5′-AAA ATC TGG GTA CGC AAA CG-3′ 5′-ACA TTA TCC GCT GGA ACA GG-3′	271
bla_{OXA-48}	5′-GCG TGG TTA AGG ATG AAC AC-3′ 5′-CAT CAA GTT CAA CC CAAC CG-3′	438

Statistical analysis

Data were analyzed using SPSS software version 20.0. Cross tabulation was used to present the relationships between data of phenotypic tests and carbapenemase gene detection among the studied isolates. Qualitative data were performed through χ^2 test and significance was set at $p \leq 0.05$. The PCR results were used as the gold standard for calculating the sensitivity and specificity of the phenotypic tests. Those for CARB compartment were for class A+B carbapenemases, and those for OXA compartment were for class D carbapenemases.

Results

Vitek-2® system identification of bacterial isolates

From various clinical specimens, 142 GNB isolates were collected.

Antimicrobial susceptibility results of carbapenemase producing Gram negative bacilli

From the total 142 GNB isolates, only 111 GNB isolates (78.17 %) were shown to be resistant to any carbapenem members (etrapenem. (ETM) 54.9 % ; imipenem (IPM) 73.9 % ; and meropenem (MEM) 72.5%) by the minimal inhibitory concentrations (MIC) using the Vitek-2® system. The detailed percentages of resistance to other antimicrobials are shown in **figure (1)**. Isolates were considered resistant to ETM if the MIC was $\geq$2 mg/L, IPM$\geq$4 mg/L, MEM$\geq$4 mg/L. Isolates were intermediate resistant if ETM 1 mg/L, IPM 2 mg/L, MEM 2 mg/L. Isolates were susceptible if ETM$\leq$0. 5 mg/L, IPM$\leq$1 mg/L, MEM$\leq$1 mg/L [18].

Phenotypic detection of carbapenemases production

Of the 111 CR GNB isolates included in the study, 101 isolates (91 %) were found to produce carbapenemase by CIM, 69 isolates (62.2 %) produced the enzyme on CARB compartment of chromID® CARBA-SMART medium and 62 isolates (55.9 %) on OXA compartment of chromID® CARBA-SMART medium giving an overall percentage of 69.4% (77 isolates) on chromID® CARBA-SMART medium. The details are shown in **table (2)**.

Genotypic detection of carbapenemase genes

Of the 111 CR GNB isolates included in the study, 91 isolates (82 %) were positive for one or more carbapenemase genes. The species distribution for isolates positive for carbapenemases genes is shown

in details in **table (2)**. The amplified genes are shown in **figure (2)** and **figure (3)**.

Correlation between phenotypic and genotypic tests for carbapenemase production

In general, there was a highly significant correlation between carbapenemase production by the phenotypic tests and the presence of carbapenemase genes (p value= < 0.0001***). Such significant correlation was specifically found in *E coli, Klebsiella pneumoniae, Enterobacter sp. and Acinetobacter baumanii* isolates as shown in **table (3)**.

Sensitivity, specificity, positive and negative predictive values of the phenotypic methods

According to our findings, OXA compartment of chromID® CARBA-SMART medium and CIM exhibited the highest sensitivity (100%), followed by CARB compartment of chromID® CARBA-SMART medium (97.3%) then overall chromID® CARBA-SMART medium (86.7 %). The specificity was greater for CARB, OXA compartments and overall chromID® CARBA-SMART medium (100%) while specificity of CIM was only 66.7 % as shown in **table (4).**

Table 2. Phenotype and genotype detection of carbapenemase producing Gram-negative bacilli.

Bacterial isolates N (%)	Carbapenem resistant by MIC N (%)	CARBA SMART medium (N , %)†			CIM	Carbapenemase genes (N, %)‡						
						Class (A)	Class (B)					Class (D)
		CARB	OXA	CARB/OXA		bla_{KPC-1}	bla_{NDM-1}	bla_{SIM-1}	bla_{VIM-2}	bla_{GIM-1}	bla_{SPM-1}	bla_{OXA-48}
E coli 29 (20.4%)	23 (20.7 %)	17 (24.6%)	15 (24.2%)	19 (24.7 %)	17 (16.8%)	8 (20 %)	10(15.4 %)	_	4 (33.3%)	_	_	4 (22.6 %)
Klebsiella pneumonia 28 (19.7%)	26 (23.4 %)	20 (29%)	16 (25.8%)	20 (26%)	26 (25.7 %)	14 (35 %)	20 (30.8%)	_	2 (16. 7%)	_	_	18 (29 %)
Acinetobacter Baumannii 22 (15.5%)	15 (13.5 %)	11 (16%)	8 (12.9%)	11 (14.3%)	15 (14.9%)	6 (15 %)	6 (9.2 %)	_	2 (16.7%)	_	2 (50 %)	8 (12.9 %)
Enterobacter sp 25 (17.6%)	17 (15.3 %)	11(16%)	13 (21%)	13 (16.9%)	15 (14.9%)	4 (10 %)	9 (13.8 %)	2(100 %)	2 (16.7%)	_	_	6 (9. 7 %)
Proteus mirabilis 10 (7.04%)	6 (5.4 %)	_	_	_	4 (4 %)	2 (5 %)	4 (6.2 %)	_	_	_	_	4 (6.5 %)
Shigella sonnei 10 (7.04%)	10 (9 %)	6 (8.7%)	8 (12.9%)	8 (10.4%)	10 (9.9%)	2 (5 %)	8 (12.3 %)	_	2 (16. 7%)	_	_	4 (6.5 %)
Pseudomonas aeruginosa 6 (4.22%)	6 (5.4 %)	_	_	_	6 (5.9 %)	2 (5 %)	4 (6.2 %)	_	_	_	_	4 (6.5 %)
Other GNB* 12 (8.5%)	8 (7.2 %)	4 (5.8%)	2 (3.2%)	6 (7.8%)	8 (7.9 %)	2 (5 %)	4 (6.2 %)	_	_	_	2 (50 %)	4 (6.5 %)
Total 142 (100%)	111	69(62.2%)#	2 (55.9 %)#	77(69.4%)#	101 (91%)#	40(36 %)#	65(58.6%)#	2(1.8%)#	2(10.8%)#	0 (0%)#	4 (3.6 %)#	62 (55.9%)#

*Other Gram-negative bacilli include *Acinetobacter haemolyticus* (4), *Achromobacter denitrificans* (4), Moraxella (2) and *Sphingomonas paucimobilis* (2).

†The percentages are calculated from the total growths on the CARBA/OXA/OR CARBA/OXA compartments

‡The percentages are calculated from the total corresponding genes

The percentages are calculated from the carbapenem resistant GNB by MIC

Table 3. Comparison between phenotypic and genotypic results of carbapenemase production among different CR GNB isolate.

| Bacterial isolates	Carbapenem resistant isolates by MIC No. (%)	Phenotypic tests (CARBA-SMART medium, CIM) Positive No. (%)	Carbapenemase gene Positive No. (%)	Correlation with carbapenemase (*p* value)
E coli	23 (20.7%)	21 (91.3%)	16(69.6%)	**<0.0001*****
Klebsiella pneumoniae	26 (23.4%)	26 (100%)	24 (92.3%)	**<0.0001*****
Acinetobacter Baumannii	15 (13.5%)	15 (100%)	10 (66.7%)	**0.014***
Enterobacter sp.	17 (15.3%)	15 (88.2%)	13 (86.7%)	**<0.0001*****
Proteus mirabilis	6 (5.4%)	4 (66.7%)	6 (100%)	**0.156**
Shigella sonnei	10 (9%)	10 (100%)	10 (100%)	**0.176**
Pseudomonas aeruginosa	6 (5.4%)	6 (100%)	6 (100%)	**0.226**
Other GNB*	8(7.2%)	8 (100%)	6 (75%)	**0.05***
Total	111(100%)	105 (94.6%)	91(82%)	**<0.0001*****

* Other Gram-negative bacilli include: *Acinetobacter haemolyticus* (4), *Achromobacter denitrificans* (4), *Moraxella* (2) and *Sphingomonas paucimobilis* (2).

Table 4. Sensitivity, specificity, positive and negative predictive values of the phenotypic methods.

	Sensitivity	Specificity	PPV*	NPV†
CARB compartment	97.3%	100%	100%	95.2%
OXA compartment	100%	100%	100%	100%
Overall CARBA-SMART medium	86.7%	100%	100%	58.8%
CIM	100%	66.7%	90.1%	100%

* PPV: Positive predictive value

† NPV: Negative predictive value

Figure 1. Antimicrobial susceptibility patterns of carbapenem resistant isolates.
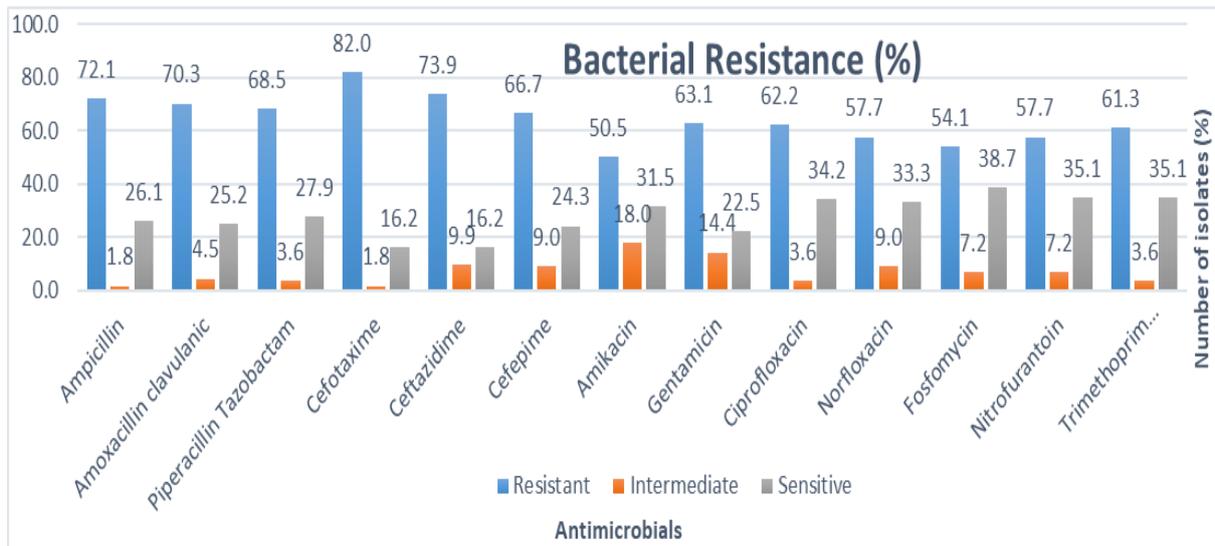
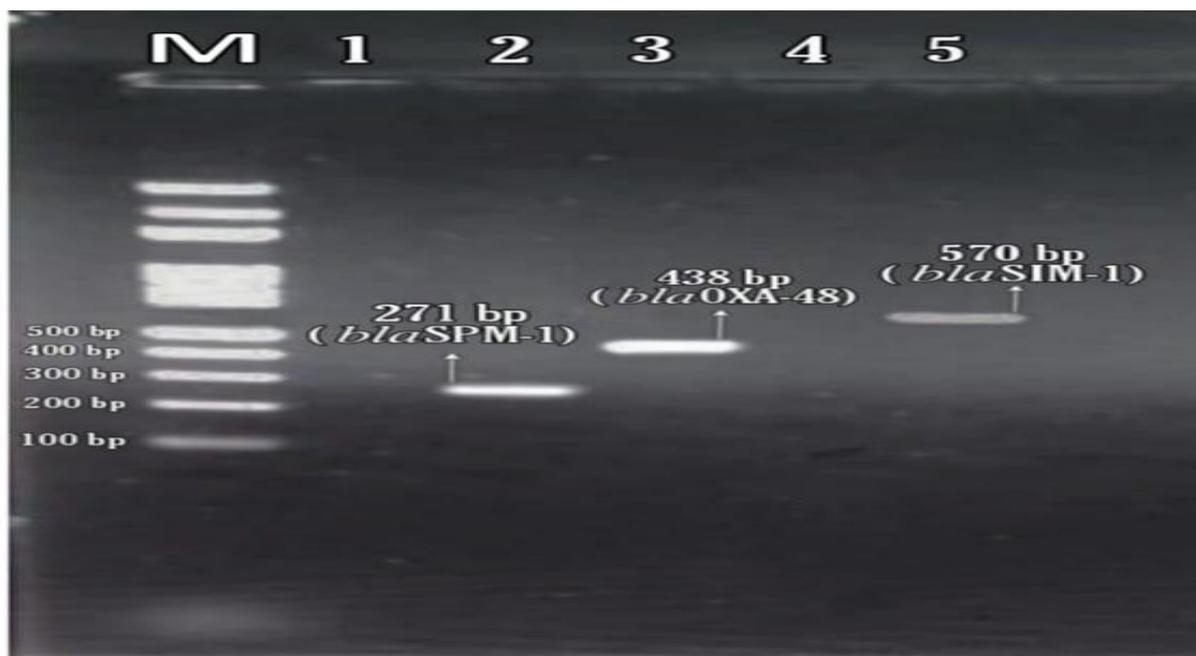(R): Resistant, (I): Intermediate and (S): Susceptible.

Figure 2. Agarose gel electrophoresis of bla$_{SPM-1}$, *bla$_{OXA-48}$* and *bla$_{SIM-1}$* genes in GNB isolates. Lane M = 100 bp DNA ladder; Lane 1= negative control; Lane 2 = positive isolate for *bla$_{SPM-1}$* (271 bp); Lane 3 = positive isolate for *bla$_{OXA-48}$* (438 bp); Lane 4 = negative isolate; and Lane 5 = positive isolate for *bla$_{SIM-1}$* (570 bp).

Figure 3. Agarose gel electrophoresis of _bla_KPC-1, bla_NDM-1, and _bla_VIM-2 genes in GNB isolates. Lane M = 100 bp DNA ladder; Lane 1= negative control; Lane 2 = _bla_KPC-1 (893 BP), Lane 3= _bla_NDM-1 (621 bp); Lane 4= _bla_VIM-2 (801 bp).

Discussion

Carbapenamases have a significant impact on the successful use of carbapenems, which are often used as the last resort. The ability of these relevant genes to be transferrable further complicates the matter. This urges the need for a low-cost and precise test for rapid identification of such enzymes to facilitate active infection control and prevention measures [1].

In this study, _E. coli_ was found to be the most frequent GNB (29/142, 20.4%), followed by _K. pneumoniae_ (n= 28, 19.7%), _Enterobacter_ sp. (n= 25, 17.6%), and _A. baumannii_ (n=22, 15.5%). These results agree with the studies of **Haji et al.** [10], **Jalalvand et al.** [19], and **Mohamed et al.** [20].

The frequency of CR GNB in this study was high reaching 78.2 % (111/142), which is higher than those reported in other Egyptian studies conducted in different governorates. The percentages of CR GNB were found to be 9.9 % in Sohag [20], 34.1% in Ismailia [21], 36 % in Cairo [22], 44.3% among _K. pneumoniae_ isolates in Suez Canal University Hospitals [23] and 62.7 % in Tanta University Hospitals [24]. The difference in prevalence could be attributed to effectiveness of infection control measures being implemented. Also, carbapenem resistance prevalence varied in different countries; 86.3% in Tunisia [9], 56% in

Pakistan [25], 13.6% and 37.9% in Iran [19], 30.9% in Iraq [10], 24.6% in China [26], 19% in Algeria [27], 5.99% in Morocco [28] 2.9% in Ghana [29].

Klebsiella pneumoniae was the most predominant CR GNB followed by _E. coli_, _Enterobacter_ sp., and _A. baumannii_ which agrees with the study of **Mohamed et al.** [20] in Egypt. Regarding the antimicrobial susceptibility profiles, carbapenem resistance has been associated with resistance to other antibiotic classes. This is in a general agreement with previous Egyptian studies, although with different percentages. **Mohamed et al.** [20] reported higher resistance rates of CR GNB to ampicillin (97%), ertapenem (91%), imipenem (97%) and meropenem (97%). On the contrary, the resistance rates in this study were higher for piperacillin/tazobactam, cefotaxime, cefepime, amikacin, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole. The resistance rate to ceftazidime was within the same range in both studies. Again, compared to the study of **Saad et al.** [30], they reported higher resistance rates against ampicillin (100%), amoxicillin/ clavulanic (92.8%), imipenem (95.2%), amikacin (76.1%) and gentamicin (80.95 %). The resistance rates of this study to cefepime and nitrofurantoin were higher than those reported by **Saad et al.** [30]. For

ceftazidime and ciprofloxacin, the resistance rates were within the same range in both studies.

Even though the phenotypic approaches identify carbapenemases differently than genotyping methods, there was a significant correlation (p <0.0001***) between them in general. The CARB compartment of chromID® CARBA-SMART medium identified 69/111 (62.2%) carbapenemase-producers, while by PCR, classes A/B carbapenemases were detected in 58.5% (83/142). Regarding the OXA compartment of the medium, 62/111 (55.9%) of the isolates were found to be carbapenemase producers, which were also genotypically detected. Overall, chromID® CARBA-SMART medium, found 77/111 (69.4%) of the isolates to be carbapenemase producers.On the other hand, the CIM detected carpapenemases in 101/111 (91%), while only 91 isolates (82 %) were positive for one or more carbapenemase genes. It is important to note that this study points to chromID® CARBA-SMART medium as potentially offering an effective method for accurately detecting OXA-48 carbapenemase genes.

As regards the performance of the different phenotypic test, the sensitivity and specificity of the chromID® CARBA-SMART medium was generally accepted being 86.7% and 100% respectively. The sensitivity and specificity of the OXA compartment were 100%, while for the CARB compartment, they were 97.3% and 100% respectively. **Willey et al.** [6] reported the same sensitivity regarding the CARB compartment (97%) but with a very low specificity (68.4%). On the contrary, they found the sensitivity of the combined agars to be much higher (99.1%). For the OXA compartment, the results of this study were somewhat higher with those of **Willey et al.** [6] who found the sensitivity and specificity to be 97% and 98.2% respectively. In accordance, the sensitivity, specificity, PPV and NPV for the chromID CARBA reported in the study of **Papadimitriou-Olivgeris et al.** [12], are in harmony with the results of this study, where they were 96.5%, 100%, 100%, 96.8% respectively. Another study concerning the CARB compartment also reported lower percentages (92.4%, 96.9% and 93.4%, 94.8 respectively) [7].

Regarding the CIM, carbapenemase production in GNB was detected with high sensitivity in this study (100 %) yet having a low specificity (66.7%). This is in agreement with **van der Zwaluw et al.** [11] who also reported a comparatively high sensitivity (98.8%) among non-

fermenters. The high sensitivity of the CIM was also previously reported (99%) with a higher specificity, PPV and NPV (98.4%, 99.0% and 98.4% respectively) compared to our results [31]. Thus, the CIM may be considered as a rapid, easy and reliable screening method that can detect carbapenemase activity in GNB in areas of limited resources to control the spread in healthcare settings. But needs the to be in conjunction with the chromID® CARBA-SMART medium which has a 100% specificity for accurate results.

In the present study, the carbapenemase genes were found in 91 of 111 (82 %) CR GNB isolates. The bla_{NDM-1} was the most prevalent gene, followed by bla_{OXA-48}, bla_{KPC-1}, bla_{VIM-2}, bla_{SPM-1}, and bla_{SIM-1} which is consistent with previous studies [10,29]. *K. pneumoniae*, *E. coli*, then *Enterobacter* sp. were the most common bacteria carrying bla_{NDM-1}. This is in consistence with previous results reporting bla_{NDM-1} to be the most prevalent in *K. pneumonia* [9,10,27,28]. For bla_{KPC-1} gene, the frequency among CR GNB was 40/111 (36%), which is consistent with studies conducted in Menoufia university [32] and Ain Shams university hospitals [33], which found that 24.07 % and 21% of isolates were positive for bla_{KPC-1}, respectively. The bla_{OXA-48} gene in this study was found in 62/111 (55.9%) of CR GNB isolates, 14/62 (22.6%) of *E. coli* isolates, and 18/62 (29%) of *K. pneumoniae* isolates. This is consistent with findings from **Khalifa et al.** [34], who found the bla_{OXA-48} gene in 49.2 % of isolates, and **Abdulla et al.** [35], who reported it in 25% of *E. coli* isolates and in 21.4 % of *K. pneumoniae* isolates. As in another study (29), *K. pneumoniae* was the most common OXA-48 producing isolates in the current study. Regarding bla_{VIM-2}, the frequency was only 10.8 % (12/111) of the CR GNB isolates. This low rate is consistent with the research of **Elbadawi et al.** [36] but differs from previous studies that showed a higher prevalence of this gene (82 %) [37]. We reported a low prevalence of bla_{SPM-1} and bla_{SIM-1} in this study (3.6 % and 1.8 % respectively) which agreed generally with previous studies but with different percentages (1.75% [38] and 4% [39] respectively). In the current study, the gene bla_{GIM-1} was not detected among CR GNB isolates, which agrees with the prior Turkish study [40].

Conclusion

The carbapenemase inhibition method(CIM) was found to be a very sensitive, easy,

and cheap test for carbapenemase detection that can be applied routinely in Microbiology laboratories with limited resources. But needs the addition of ChromID® CARBA-SMART medium to improve the performance. This study has a high prevalence of carbapenemases among CR GNB isolates with the potential of rapid spread necessitating the need for the phenotypic tests. The CR GNB generally had high percentages of resistance to many antibiotic classes The most prevalent carbapenemase genes were the bla_{NDM-1} and bla_{OXA-48} detected mostly among CR *K.* pneumonia isolates

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