Microbes and Infectious Diseases 2023; 4(3): 853-870

Microbes and Infectious Diseases

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

Original article

Phenotypic and genotypic characterization of carbapenemases in carbapenem-resistant Gram-negative bacilli isolated from adult cancer patients

Dalia El.Nobi [*1], Sherein G. Elgendy [2], Rania Bakry [3], Abeer S. Hassan [4], Ehsan M.W. El-Sabaa [1]

1- Department of Microbiology & Immunology, Faculty of Pharmacy, Assiut University, Assiut, Egypt.
2- Department of Medical Microbiology & Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.
3- Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt.
4- Department of Pharmaceutics, Faculty of Pharmacy, South Valley University, Egypt.

ARTICLE INFO

Article history:
Received 1 July 2023
Received in revised form 9 July 2023
Accepted 14 July 2023

Keywords:
Multidrug-resistant
Carbapenem resistance
NDM
OXA-48
Cancer patients

ABSTRACT

Background: Carbapenem-resistant Gram-negative bacteria (CR-GNB) infections are prevalent in cancer patients with weakened immune systems, causing significant morbidity and mortality. The empirical use of antimicrobials has reduced mortality but led to the emergence of multidrug-resistant (MDR) bacteria. In this study, identification and susceptibility testing were carried out using standard procedures (Kirby-Bauer and broth microdilution techniques), and phenotypic and genotypic detection of carbapenemase-producing GNB isolated from adult cancer patients was performed using conventional procedures. **Methods:** One hundred and eight Gram-negative bacteria were recovered from various specimens, with the most common isolates being, *Escherichia (E.) coli (45; 41.7%),* followed by *Klebsiella* spp. *(38; 35.2%), Acromobacter* spp. (9;8.3%), *Acinetobacter (A.) baumannii* (5; 4.6%) and others including *Enterobacter aerogenes, Raoultella ornithinolytica, Serratia fonticola, Citrobacter brakii, Comamonas testosteroni, Proteus mirabilis* (11; 10.2%). Concerningly, 64 of 108 Gram-negative bacterial isolates (59.3%) were MDR. Furthermore, 91 out of 108 GNB isolates (84.3%) revealed a pattern of meropenem resistance using the broth microdilution method, which is a worrying rise in the rate of carbapenem resistance. Following the modified carbapenem inactivation method (mCIM), EDTA carbapenem inactivation method (eCIM), and combined disc test as phenotypic tests for the preliminary screening of carbapenemase producers (CPs), conventional PCR was performed on the 91 extracted DNA (Using 6 common carbapenemase primers). **Results:** It was found that bla*NDM* was the most common 60(66%), then bla*OXA-48*, *VIM* 47 (51.6%), bla*IMP* 32(35.2%), bla*KPC* 20(22.2%), and bla*GES* 12(13.2%). **Conclusion:** Based on these results, rapid and precise carbapenemase detection is crucial for clinical care, epidemiological investigations, and infection control.

DOI: 10.21608/MID.2023.219321.1556
* *Corresponding author:* Dalia El.Nobi
E-mail address: daliaelnobi75@yahoo.com

Introduction

Millions of deaths each year are attributed to infections with multidrug-resistant bacteria (MDRB), making them a serious threat to public health [1]. Multidrug-resistant bacteria colonization is a major warning sign for developing subsequent MDRB infection [2, 3]. Prolonged hospital stay and antibiotic therapy are considered triggers for colonization, making cancer patients a population that is vulnerable to MDRB colonization [4], and cancer patients often exhibit these factors [5]. Gram-positive bacteria (GPB) have dominated the bacterial pathogen population in cancer patients for several decades. yet, new studies have shown an epidemiological switch among numerous cancer treatment facilities, with the resurgence of GNB as prevalent bacteria in this type of setting[6, 7].

Vancomycin-resistant *enterococci* (VRE) and CR-GNB colonization are of special concern because these bacteria have been associated with more disastrous outcomes than other MDRB, such as enterobacteria that produce extended-spectrum beta-lactamases(ESBLs) [8]. The prevalence of CR-GNB infections has steadily increased as a result of the overuse and improper application of carbapenem antibiotics [9] a result of mobile genetic elements carrying beta-lactamases genes [10].

The World Health Organisation (WHO) identified the pathogenic organisms of major threat in the global priority list of antibiotic-resistant bacteria as carbapenem-resistant *Enterobacterales* (CRE), carbapenem-resistant *Acinetobacter baumannii* (CRAB), and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), to guide research, the discovery, and the generation of novel antibiotics in 2017[11].

Among the top six pathogens for resistance-related deaths are *Klebsiella pneumoniae, A. baumannii, P. aeruginosa, and E. coli*. CRAB and carbapenem-resistant *K. pneumoniae* (CRKP) are among the top seven MDR pathogens, each of which is responsible for more than 50,000 deaths linked to antimicrobial resistance [1]. Carbapenems are frequently the last class of antibiotics still in use to treat infections caused by MDR-GNB[12, 13].

The most frequent causes of carbapenem resistance are acquired class A (*KPC, IMI, GES*), class B (*IMP, VIM, NDM*), and class D (*OXA-48, OXA-181*) carbapenemases[14, 15]. The spreading and transmission of the supporting genes are encouraged by their frequent associations with mobile genetic structures (such as plasmids, integrons, and transposons). Because of the high mortality rates among infected patients, carbapenemase-producing gram-negative bacteria (CP-GNB) are regarded as an urgent threat[16, 17].

Rapid detection of CPs is critical for treating this worrisome public health concern because of elevated fatality rates, significant antibiotic resistance that restricts options for therapy, and the great potential for worldwide spread [18]. Consequently, the goal of our study was to use phenotypic testing in addition to molecular techniques to establish the prevalence of CP-GNB in adult cancer patients.

Patients and methods

Ethical statement

The study protocol was reviewed and approved by the Committee of Medical Ethics of the Faculty of Medicine, Assiut University, Egypt (IRB NO:17101590). Informed and written consent was obtained from all patients after explaining the study's purpose.

Patients

This cross-sectional study was carried out in the Department of Internal Medicine, Hematology Unit, and the Department of Clinical Pathology at South Egypt Cancer Institute, Assiut University, Assiut, Egypt. Inclusion criteria were all the admitted adult patients for the treatment of cancer and diagnosed with a Gram-negative bacterial infection from January 2022 to January 2023. A total of 2648 patients were admitted to the Department of Internal Medicine, Hematology Unit, and the microbiology laboratory, Department of Clinical Pathology at South Egypt Cancer Institute during the study period. 348 patients were adult cancer patients diagnosed with infections. Out of these, 108 patients had documented Gram-negative bacterial infections.

Methods

Collection and examination of bacterial isolates

One hundred and eight Gram-negative bacterial isolates were recovered from urine cultures, blood cultures, sputum cultures, pus, rectal swabs, throat swabs, stool, and paracentesis from 108 adult cancer patients. Microscopic examination, culture procedures, and biochemical tests were used to detect, isolate, and identify pathogens in the specimens [19] and confirmed by VITEK 2 system (Model compact, Biomerieux, USA).

Antibiotic sensitivity testing by disc diffusion method

Antibiotic susceptibility testing for commonly used antimicrobials like imipenem (10 mg), meropenem (10 mg), ceftriaxone (30 mg), ciprofloxacin (5 mg), and amikacin (30 mg) was carried out on using the Kirby-Bauer method. Isolates with resistance to at least one agent in three or more antibiotic categories were identified as MDR[20]. VITEK AST validated the results, and the antibiotic names and standard inhibition diameters were utilized following CLSI guidelines, 2020 [21]. As a quality control, the reference strain *E. coli* ATCC 8739 was employed.

Carbapenem susceptibility testing and determination of minimum inhibitory concentration (MICs)

The carbapenem susceptibility pattern of isolates to meropenem was tested for their MICs against meropenem using the broth microdilution method in 96 multi-well microtiter plates according to the CLSI 2020 reference standards. Isolates with resistance patterns to meropenem were regarded as carbapenem-resistant[22]. As a quality control, *E. coli* ATCC 8739 was employed.

Phenotypic detection of carbapenemase producers (CPs)

To perform phenotypic testing, two methods the mCIM and eCIM test and the combined disc test were employed.

Modified carbapenem inactivation method (mCIM) and EDTA carbapenem inactivation method (eCIM) methods.

On isolates that were not susceptible to at least one carbapenem, a modified carbapenem inactivation method was employed. Briefly, two tubes containing 2 ml of tryptic soy broth (TSB) are resuspended with a 1µl loopful of isolates that are carbapenem-resistant with probable carbapenemase activity. While the second tube (eCIM) had EDTA with a final concentration of 5 mM, the first tube (mCIM) did not contain any EDTA at all. Each tube contained a 10 µg meropenem disc, which was incubated for 4 h ±15 min at 35ºc in ambient air. The discs were removed and plated on Mueller-Hinton agar (MHA) plates that had just been plated with a 0.5 McFarland suspension of the *E. Coli* ATCC 8739 strain. After incubation for 18-24hr, the results were interpreted [23].

Combined disc test

Combined discs of meropenem alone and with those of phenylboronic acid (PBA) or ethylene diamine tetra acetic acid (EDTA) or both PBA and EDTA were used to detect the production of carbapenemase and to differentiate the *KPC* and MBLs enzymes. The diameter of the growth inhibitory zone around the meropenem disc in the presence of PBA, EDTA, and PBA+EDTA was compared to that of the plain meropenem disc[24].

Polymerase chain reaction (PCR) amplification of carbapenemases genes

According to **Junior et al.** using the boiling technique, total DNA was extracted from bacterial isolates [25]. The extracted DNA was spectrophotometrically quantified using a Nanodrop spectrophotometer (Epoch, USA). The detection of genes encoding carbapenemase was performed in a thermocycler (SensoQuest Labcycler, Germany) using specific oligonucleotide primers **(Table 1).** The PCR product was visualized after agarose gel electrophoresis.

Statistical analysis

SPSS (Statistical Package for the Social Science, version 20, IBM, Armonk, New York) was used to gather and analyze the data. To investigate whether the data adhered to a normal distribution, the Shapiro test was applied. Quantitative data with normal distribution are expressed as mean ± standard deviation (SD) and compared with Student T -test (two different means) and ANOVA test (> two different means). Numbers (n) and percentages (%) are used to represent nominal data. The receiver operator characteristics (ROC) curve was used to compare the MEM susceptibility pattern by disc diffusion methods to the MIC by broth microdilution method and the accuracy of the combined disc test and mCIM in predicting carbapenemase production to the PCR as the gold standard test. The level of confidence was maintained at 95%, so. *p* value of less than 0.05 was deemed significant.

Table 1. PCR primers for target genes and their sequence.

Primer	Sequence (5′ to 3′)	Amplicon Size (base pair) (bp)	Annealing temperature	Gene	Reference
NDM-1F *NDM*-1R	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	621	57	bla*NDM-1*	[26]
*OXA-48*F *OXA-48R*	TTGGTGGCATCGATTATCGG GAGCACTTCTTTTGTGATGGC	744	58	bla*OXA-48*	[27]
KPC F *KPC* R	ATTTTCAGAGCCTTACTGCCC TATCGTTGATGTCACTGTATCG	901	55	bla*KPC*	[28]
GES F *GES* R	ATGCGCTTCATTCACGCAC CTATTTGTCCGTGCTCAGG	864	60	bla*GES*	[27]
IMP F *IMP* R	GGAATAGAGTGGCTTAAYTC TCGGTTTAAYAAAACAACCACC	232	55	bla*IMP*	[26]
VIM F *VIM* R	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390	55	bla*VIM*	[26]

Results

Demographic and clinical profiles of cancer patients

One hundred and eight adult cancer patients with a mean age of 51 ± 17.26 (years) with a range between 18 and 91 years old were studied. As seen in **table (2)** sixty-three (58.3%) were males, The most frequent source of malignancy was hematological (28.7%), genito-urinary tract (27.8%), and gastrointestinal tract (25.9%) malignancies, respectively.

Frequency and percentage of isolated GNB from collected specimens

Out of 108 GNB that were obtained and recovered from various clinical specimens during the study, The most frequently isolated bacteria were *E. coli* (41.7%) and *Klebsiella* species (35.2%), and the least isolated were *Citrobacter brakii*, *Comamonas testosteroni*, and *Proteus mirabilis* in one patient according to **table (3).**

Specimen type

Of a total of 108 specimens,45 (41.7%) was urine, 23(21.3%) blood, 19(17.6%) sputum and 12(11.1%) pus, 5(4.6%) rectal swab, 2(1.9%) throat swab, 1(0.9%) stool and 1(0.9%) ascetic fluid sample.

Antibiotic sensitivity testing by disc diffusion method

Following CLSI guidelines 2020, the Kirby-Bauer disc diffusion method was used to test the antibiotic susceptibility of isolated GNB. The highest resistance was found against ceftriaxone (CTR) 98.1% (106/108), followed by ciprofloxacin (CIP) 91.7% (99/108), meropenem (MEM) 60.2% (65/108), amikacin (AK) 54.6% (59/108), and the least resistance was observed against imipenem (IMP) 36.1% (39/108).

Prevalence of MDR bacterial isolates based on age group, sex, and types of isolates.

For each isolate, MDR level was assessed, out of 108 bacterial isolates, 59.3% (64/108) were MDR bacteria. Twenty (18.5%) isolates were PDR, which were resistant to all antibiotics employed in our study. **Table 4** shows the level of resistance based on age groups, sex, and types of isolates respectively.

It was found that the age group of 40-59 years had the highest frequency of MDR (35.9%) and PDR (45%) with no significant difference (p= 0.36). Also, MDR and PDR were frequently present in males (56.3% vs. 55%; respectively) in comparison to females (43.8% vs. 45%; respectively) with no significant difference (p= 0.56).

The highest frequency of bacterial isolates with MDR was found in *E. coli* (42.2%; 27/64), followed by *Klebsiella* species (31.3%; 20/64) while the highest frequency of bacterial isolates with PDR was found in *Klebsiella* species (55%; 11/20) followed by *E. coli* (25%; 5/20), with no significant difference (p=0.39).

Carbapenem susceptibility testing and determination of minimum inhibitory concentration (MICs)

Meropenem resistance was found in 91 (84.3%) isolates, and meropenem sensitivity was found in 17 (15.7%), according to the MIC by broth microdilution method findings. By using disc diffusion and the broth microdilution method, the prevalence of meropenem resistance was assessed, as well as the screening of potential CPs.

Accuracy of MEM susceptibility pattern by disc diffusion methods as compared by MIC by broth microdilution method.

It was found that MEM susceptibility test by disc diffusion had 100% sensitivity, and 40% specificity with 76.1% overall accuracy in the prediction of MEM susceptibility pattern as compared to MIC by broth microdilution method as more accurate method with an area under the curve (AUC) was 0.698 with $p< 0.001$ as shown in **table (5)** and **figure (1).**

Phenotypic detection of carbapenemase producers

mCIM and eCIM test

The mCIM method was used to identify 82 (90.1%) of the 91 isolates of carbapenem-resistant bacteria as carbapenemase-producing isolates. Comparing the results of mCIM and those of eCIM revealed that 20 (24.4%) isolates were detected as serine carbapenemase-producing isolates, and 62 (75.6%) out of 82 isolates were metallo β-lactamase (MBLs) producers as shown in **figure (2I).**

Combined disc test

Only 69 (75.8%) of the 91 carbapenem-resistant isolates produced *KPC*, MBL, and *KPC*+MBL enzymes, and 22 (24.2%) of the isolates tested negative for both *KPC* and MBL using the combined disc test. Among the 69 carbapenemase-producing isolates, 9.9% (9/91) produced *KPC*, 35.2% (32/91) produced MBLs, and 30.7% (28/91) produced both *KPC* and MBLs as shown in **figure (2II).**

Detection of carbapenemases genes by conventional PCR

Carbapenem-resistant isolates were screened for six carbapenemase genes by PCR, and the result of PCR revealed that 8 (8.8%) isolates haven't any one of those genes while the other 83 (91.2%) isolates had either single (17.5%) or more than one gene (73.7%). The most frequent genes were *NDM* (66%), *OXA-48* (51.6%), and *VIM* (51.6%) as shown in **table (6)** and **figure (3).** Also, dual coexistence of genes were frequently found among the isolates and the most commonly detected genes were *OXA-48*&*NDM* (40.7%) and *NDM*&*VIM* (35.2%). Triple coexistence in the form of *OXA-48+GES+KPC* and *NDM+VIM+IMP* was found in 3 (3.3%) and 13 (14.3%) isolates; respectively as shown in **table (6)** and **figure (4).**

Escherichia coli ATCC® 8739 TM* reference strain was positive for bla *OXA-48* and bla *IMP* gene, *Klebsiella pneumoniae* ATCC® 33495 TM* was positive for bla *OXA-48*, bla *VIM* and bla *IMP* gene. **Figure 5** shows gel electrophoresis of carbapenemases genes.

Accuracy of combined disc test and mCIM in the prediction of carbapenemase production compared to PCR as a gold standard.

It was found that the combined disc test has 94% sensitivity and, 50% specificity with 90.1% overall accuracy in the prediction of carbapenemases(CPases) production with an area under the curve was 0.720 while mCIM has 78% sensitivity and, 50% specificity with 75.5% overall accuracy in prediction of CPases production with an area under the curve was 0.642 as shown in **table (6)** and **figure (3).**

Table 2. Demographic and clinical profiles of cancer patients.

	N= 108
Age (years)	51 ± 17.26
Range	18-91
Age groups	
18-39 years	27 (25%)
40-59 years	41 (38%)
≥ 60 years	40 (37%)
Sex	
Male	63 (58.3%)
Female	45 (41.7%)
Site of malignancy	
Hematological malignancy	31 (28.7%)
Genito-urinary tract	30 (27.8%)
Gastrointestinal tract	28 (25.9%)
Breast cancer	5 (4.6%)
Sarcoma	4 (3.7%)
Lung cancer	3 (2.8%)
Thyroid cancer	1 (0.9%)
Unknown primary	6 (5.6%)

Data expressed as frequency (percentage), mean (SD), and range.

Table 3. Frequency and percentage of isolated Gram-negative bacteria from collected specimens.

Isolated bacteria	**N= 108**
E. coli	45 (41.7%)
Klebsiella species	38 (35.2%)
Acromobacter species	9 (8.3%)
Acinetobacter baumanii	5 (4.6%)
Enterobacter aerogenes	4 (3.7%)
Raoultella ornithinolytica	2 (1.9%)
Serratia fonticola	2 (1.9%)
Citrobacter brakii	1 (0.9%)
Comamonas testosteroni	1 (0.9%)
Proteus mirabilis	1 (0.9%)

Data expressed as frequency (percentage).

Table 4. Level of resistance based on age group, sex, and types of isolates.

	Level of resistance		***P* value**
	MDR (n= 64)	**PDR (n= 20)**	
Age groups			0.36
18-39 years	20 (31.3%)	3 (15%)	
40-59 years	23 (35.9%)	9 (45%)	
≥ 60 years	21 (32.8%)	8 (40%)	
Sex			0.56
Male	36 (56.3%)	11 (55%)	
Female	28 (43.8%)	9 (45%)	
Isolated bacteria			0.39
E. coli	27 (42.2%)	5 (25%)	
Klebsiella species	20 (31.3%)	11 (55%)	
Acromobacter species	7 (10.9%)	1 (5%)	
Acinetobacter baumanii	2 (3.1%)	1 (5%)	
Enterobacter aerogenes	2 (3.1%)	1 (5%)	
Raoultella ornithinolytica	2 (3.1%)	0	
Serratia fonticola	2 (3.1%)	0	
Citrobacter brakii	0	1 (5%)	
Comamonas testosteroni	1 (1.6%)	0	
Proteus mirabilis	1 (1.6%)	0	

Data expressed as frequency (percentage). *P* value was significant if < 0.05. MDR: multi-drug resistance; PDR: pan-drug resistance.

Table 5. Accuracy of MEM susceptibility pattern by disc diffusion method.

Indices	**Value**
Sensitivity	100%
Specificity	40%
Positive predictive value	71.4%
Negative predictive value	100%
Accuracy	76.1%
Area under curve	0.698
P value	< 0.001*

P value was significant if < 0.05.

Table 6. Detection of carbapenemase genes in carbapenem-resistant isolates.

Carbapenemase genes	**N= 91**
NDM	60 (66%)
OXA-48	47 (51.6%)
VIM	47 (51.6%)
IPM	32 (35.2%)
KPC	20 (22.2%)
GES	12 (13.2%)
Dual coexistence	
OXA-48+NDM	37 (40.7%)
OXA-48+VIM	23 (25.3%)
OXA-48+IMP	14 (15.4%)
OXA-48+GES	6 (6.6%)
OXA-48+KPC	10 (11%)
NDM+VIM	32 (35.2%)
NDM+IMP	20 (22%)
NDM+GES	8 (8.8%)
NDM+KPC	11 (12.1%)
VIM+IMP	23 (25.3%)
VIM+GES	9 (9.9%)
VIM+KPC	14 (15.4%)
IMP+GES	8 (8.8%)
IMP+KPC	13 (14.3%)
GES+KPC	9 (9.9%)
Triple coexistence	
OXA-48+GES+KPC	3 (3.3%)
NDM+VIM+IMP	13 (14.3%)

Data expressed as frequency (percentage).

Table 7. Accuracy of combined disc test and mCIM in the prediction of CPase production.

Indices	**mCIM**	**Combined disc test**
Sensitivity	78%	94%
Specificity	50%	50%
Positive predictive value	94%	95%
Negative predictive value	18%	44%
Accuracy	75.5%	90.1%
Area under curve	0.642	0.720
P value	0.145	0.021

P value was significant if < 0.05.

Figure 1. ROC curve MEM susceptibility pattern by disk diffusion method.

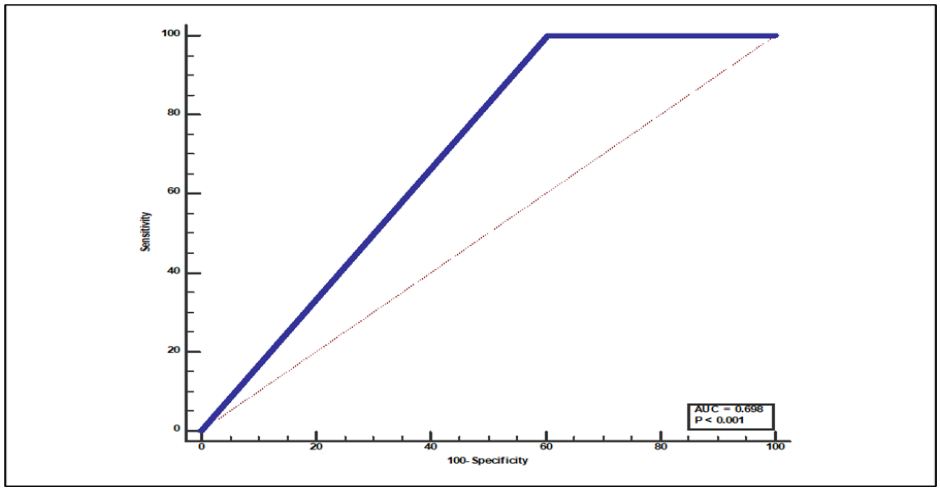


Figure 2. I) Phenotypic mCIM and eCIM A) Serine carbapenemases-producing isolates; B) MBLs-producing isolates; **C)** non-carbapenemases-producing isolates **II) Phenotypic Combined disc test A)** Serine carbapenemases-producing isolates**; B)** MBLs producing isolates**; C)** non-carbapenemases-producing isolates..

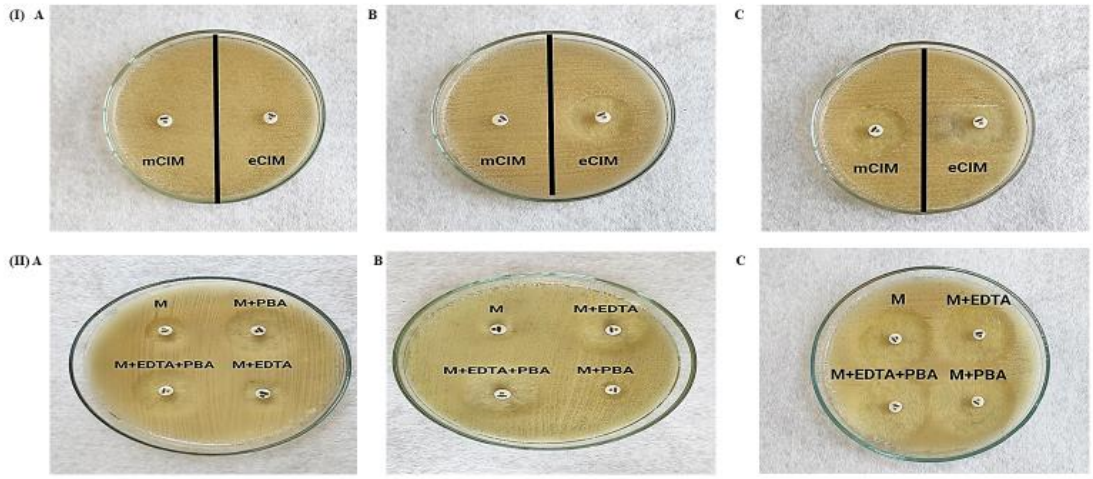


Figure 3. Detection of carbapenemase genes in carbapenem-resistant bacterial isolates.

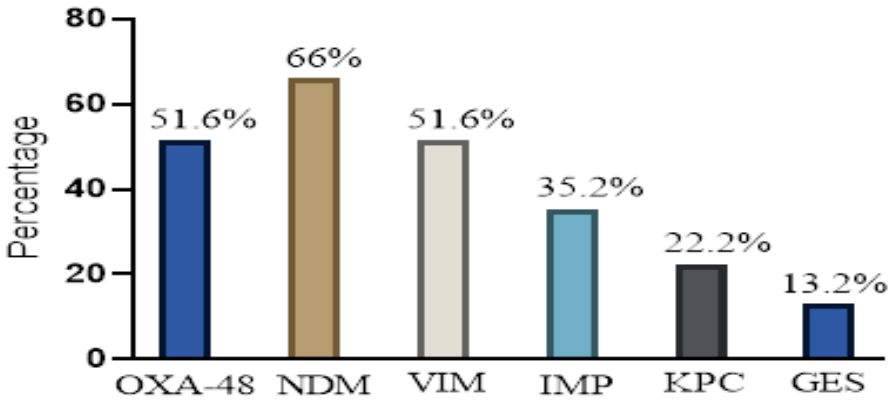


Figure 4. Coexistence of carbapenemase genes in carbapenem-resistant bacterial isolates.

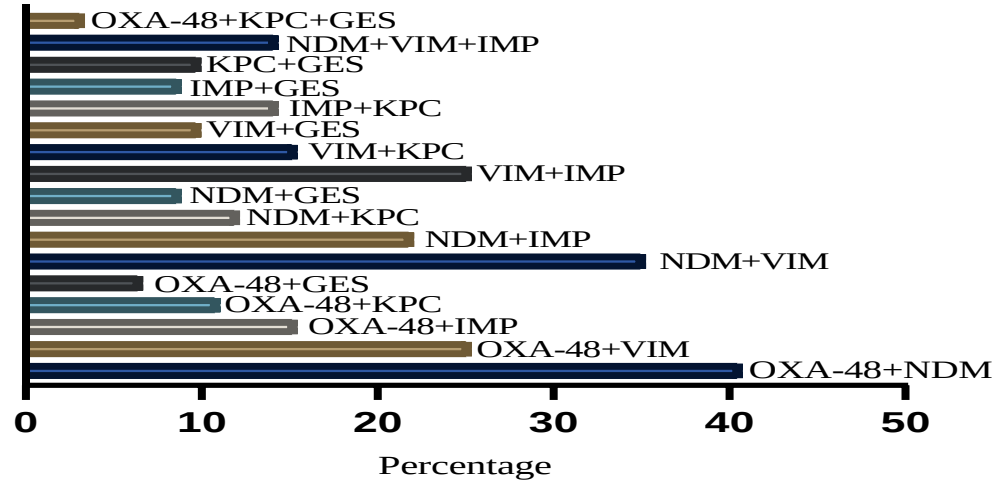


Figure 5. Agarose gel electrophoresis of **I)** *OXA-48* gene product (amplified size 744 bp) PC: positive control *Escherichia coli* ATCC® 8739 TM* reference strain, Lane 1&2: positive results for the gene, Lane 3&4: negative results for the gene; **II)** *VIM* gene product (amplified size 390 bp). Lane (M), molecular size marker of DNA (100-1500bp Ladder). PC: positive control *Klebsiella pneumoniae* ATCC® 33495 TM* reference strain, Lane 1&2: positive results for the gene, Lane 3&4: negative results for the gene**;III)** *IMP* gene product (amplified size 232 bp). Lane (M), molecular size marker of DNA (100-1500bp Ladder). PC: positive control *Escherichia coli* ATCC® 8739 TM* reference strain, Lane 1: positive result for the gene, Lane 2-5: negative results for the gene; **IV)** *NDM* gene product (amplified size 621 bp). Lane (M), molecular size marker of DNA (100-1500bp Ladder), Lane 1 -3: positive results for the gene, Lane 4&5: negative results for the gene; **V)** *KPC* gene product (amplified size 901 bp). Lane (M), molecular size marker of DNA (100-1500bp Ladder). Lane 1 -3: positive results for the gene, Lane 4-6: negative results for the gene**; VI)** *GES* gene product (amplified size 864 bp). Lane (M), molecular size marker of DNA (100-1500bp Ladder). Lane 1 -3: positive results for the gene, Lane 4-6: negative results for the gene.

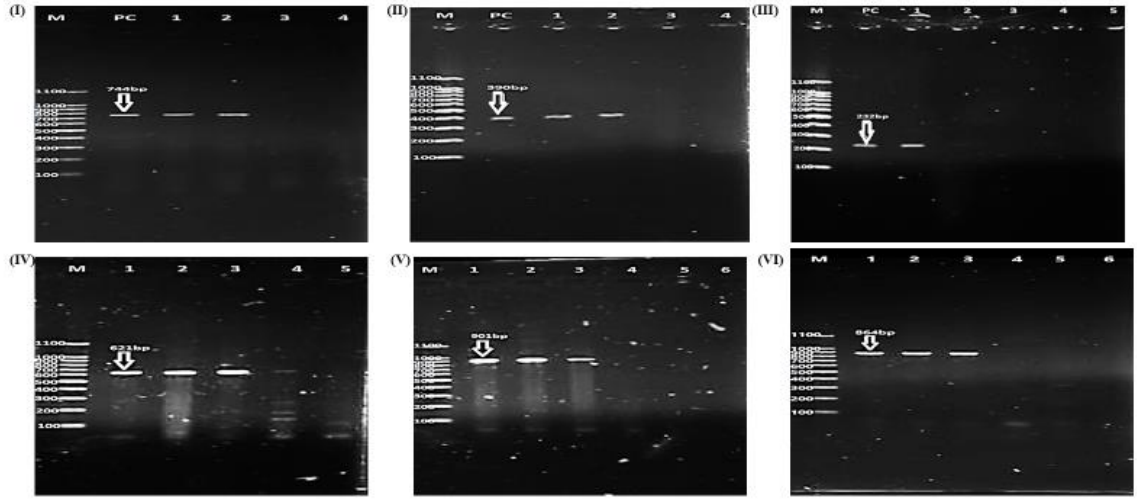
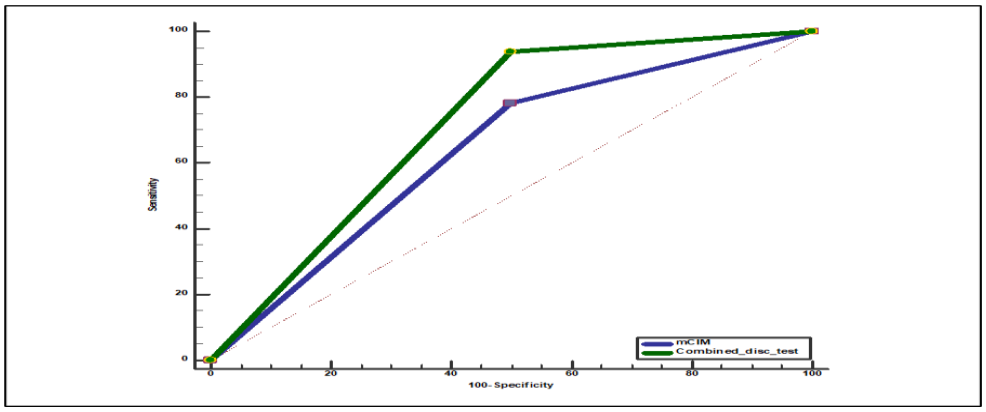


Figure 6. Accuracy of combined disc test and mCIM in the prediction of CPase production compared to PCR as a gold standard.



Discussion

Infections are still a major source of substantial morbidity and mortality in cancer patients. Gram-negative bacteria have dominated the scene as the main source of infections in cancer patients [29]. *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *A. baumannii* are Gram-negative bacteria that have been more frequently linked to cancer patients[30].

Haematological (28.7%) and genitourinary (27.8%) malignancies were the most prevalent sources of malignancy in our study. In the study of **Lakshmaiah et al.** lymphoma, leukemia, and germ-cell tumors were the most prevalent sources of malignancy[31]. According to **Tamai et al.** blood malignancies are the most prevalent type of malignancy[32].

Forty-one point seven (41.7%) and 21.3%, respectively, of the recovered Gram-negative bacterial isolates in our study were from urine and blood cultures. Approximately 100% of patients in various studies reported having bacteremia[33, 34]. The frequency of positive urine and blood cultures was 5% and 8%, respectively, in the study by Soroush et al.[35].

Among a total of 108 bacterial isolates, the most frequently isolated bacteria were *E. coli*, *Klebsiella* spp., and *Acromobacter* spp. respectively. The microbiological findings of our study were consistent with those of previous studies that highlight the ongoing dangers posed by GNB, particularly *E. coli*, and *Klebsiella* spp. in leukemic hosts [36, 37].

Utilizing the Kirby Bauer diffusion method, our study identified the antibiotic sensitivity pattern of each isolate. Based on this, ceftriaxone is the antibiotic that is most commonly resistant (98.1%), followed by ciprofloxacin (91.7%), meropenem (60.2%), and amikacin (54.6%), all of which have more than 50% of the resistance. This result is consistent with a study conducted by **Babypadmini et al.** that found 91.6% of ESBL-producing *E. coli* bacteria to be resistant to fluoroquinolones using the disc diffusion method [38]. In contrast to our study, a lower rate of ciprofloxacin resistance was reported [39-41].

In our study, MDR GNB isolation rates were higher (59.3%), which may be related to variances in antibiotic policy or indiscriminate consumption of antibiotics in some cases in our hospital. This result is consistent with earlier data from Germany University Hospital, where 143 rectal swabs from refugees were taken, and 60.8% of those were positive for MDR GNB[42]. In Ethiopia, a higher frequency ($\geq$ 70%) of fermentative GNB recovered from ICU patients was found[41]. In contrast, the ICUs at Germany University Hospital had a lower prevalence of MDR infections [43] and Mexico cancer center [44] recorded 33.8%, and 39.5% among 325 and 266 isolated bacteria, respectively.

The major driving factor for the rapid evolution of MDR is lateral gene transfer, which is controlled by a variety of mobile genetic elements. Transposons and/or plasmids frequently contain integrons that promote the spread of resistance genes [45].

Multidrug-resistant (MDR) bacterial infections, particularly those caused by CR-GNB, are on the rise and pose a serious threat to the health of the public since they are related to elevated incidence of cancer patients morbidity and mortality [46].

In our study, we used the more sensitive broth microdilution method in addition to disc diffusion to assess carbapenem resistance. By using the disc diffusion method and the broth microdilution method, respectively, the results showed that 65 (60.2%) and 91 (84.3%) of the isolates were meropenem resistant [47]. This percentage of resistance (84.3%) exceeds the percentages reported in recent Egyptian research documenting the isolation of CRE, which were 46%[48], 68.8% [49] 59%[50], from patients with malignancy, and 48% from ICUs[51]. In the study conducted by **Hassuna et al.** it was found that 95% of the *K. pneumoniae* isolates were resistant to carbapenem [52]. The study period, area, and laboratory technique used may be the causes of this discrepancy.

Since the outbreak of CR-GNB some years ago, they have become one of the top causes of death among hospital-acquired infections [53]. This has raised concern about developing reliable and quick methods for detecting carbapenemases using phenotypic or genotypic approaches, which are a major driver of carbapenem resistance dissemination [54]. In our study, we attempted to compare the two phenotypic techniques of combined disc test, eCIM, and mCIM for carbapenemase detection in CR-GNB. Additionally, conventional PCR was carried out as a reference technique to identify six genes encoding carbapenemase [55, 56].

Carbapenemase detection can be challenging since the magnitude of carbapenem resistance triggered by carbapenemase expression alters and no one phenotypic test can be considered adequate for all circumstances [57].

In our study, we evaluated all 91 isolates of carbapenem-resistant bacteria, and the mCIM technique identified 82 (90.1%) of them as carbapenemase-producing isolates. A comparison of the mCIM and eCIM results revealed that 62 (75.6%) of the 82 CPs are MBL producers. However, The failure of the eCIM assay to discriminate between serine and MBL carbapenemase production in isolates expressing both enzymes is one of its drawbacks [23].

Using eCIM, 62 isolates were identified as MBL producers in our study. Moreover, eCIM demonstrated great accuracy in identifying 58 isolates as MBLs (*NDM*, *VIM*, and *IMP*) carbapenemase producers when results were compared to those from PCR.; however, 35 isolates possessed both MBLs and serine carbapenemases, and twenty isolates were identified as serine carbapenemase producers using eCIM. likewise, comparing eCIM and PCR findings showed that seventeen isolates possessed both MBLs and serine carbapenemases. As a result, mCIM and eCIM are highly specific in circumstances where the isolate solely produces MBLs or serine carbapenemases.

In our study, The dual application of the inhibitors, PBA and EDTA, to detect MBL and KPC coproduction. Employing both inhibitors appears to weaken both carbapenemases activity against meropenem, making it possible to identify isolates that co-produce these enzymes almost all the time.

Of these 91 carbapenem-resistant bacterial isolates, 69(75.8%) isolates were producing *KPC*, MBL, and *KPC*+MBL enzymes. Twenty-two isolates were negative for *KPC*, MBL, and both by combined disc test. Among the 69 carbapenemase-producing isolates, 9 (9.9%) produced *KPC*, 32 (35.2%) produced MBL, and 28 (30.7%) produced both *KPC*+MBL. According to a study by **Bansal et al.** and **Baraniak et al.** *KPC* producers were more prevalent than MBL producers, but in our study, MBL producers were more prevalent. [58, 59].

In *Enterobacteriaceae*, MBLs and *KPC* are regarded as significant hazards, and they may be the cause of clinical failure in approximately all β-lactam antibiotic-treated patients [15, 60]. Additionally, *KPC* and MBL genes are frequently co-transferred with ESBL, fluoroquinolone, and aminoglycoside resistance genes via plasmids[15, 61-63]. In our study, the co-production of MBL and *KPC* was 30.7%, which was consistent with the findings of **Tsakris et al.** who found the co-production of MBL and *KPC* to be 21.98%[24]. Co-production of both enzymes may contribute to their hydrolytic activity and degrees of resistance to broad-spectrum β-lactams, as well as their possible co-migration.

In our study, carbapenemase genes were detected using a polymerase chain reaction in meropenem-resistant GNB. One or more carbapenemase genes were found in 83 (91.2%) of the 91 isolates tested. bla*NDM* was the most common gene in the isolates (66%), followed by *OXA-48* and *VIM* (51.6%).

Several studies used various genotypic approaches to investigate the prevalence of carbapenemase genes. One hundred and three (71.53%) carbapenem-resistant organisms were

found to have carbapenemase genes by **Rudresh et al.** using PCR. *NDM* (52%), *OXA-48* (28%), multiple genes (20%), and *VIM* (3%), with no *KPC* or *IPM* genes found, represent the distribution of carbapenemase genes[64]. The most often identified genes were *KPC* and *NDM*, which were present in 19 (38%) of the isolates in Egypt, where **ERFAN et al.** utilized PCR to identify carbapenemase genes in 42 meropenem-resistant GNB (84%)[65]. **El Naggar** observed that 28 (75.7%) of the 37 carbapenem-resistant isolates and confirmed the production of carbapenemases using phenotypic techniques were also positive for carbapenemase genes via multiplex PCR; *KPC* 6 (21.4%), *NDM* 9 (31.2%), *OXA* 7 (25%), *IMP* 5 (17.9%), and *VIM* 1 (3.6%)[66]. The carbapenemase gene distribution varies according to geographical region, antimicrobial agent use, the pattern of commonly found pathogens, and preventative methods for infection control. Since the genes that encode carbapenemase are typically found on plasmids, this resistance mechanism has a higher likelihood of spreading[67]

The high prevalence of the *NDM* gene due to the plasmids carrying *blaNDM*-1 is a versatile gene that can incorporate a large number of other resistance genes (for example, ESBL-alleles) as well as other carbapenemase genes such as bla*OXA-48* and bla*VIM*. These plasmids were thought to be the cause of multidrug resistance in a single bacteria[68, 69].

The elevated level of *OXA-48* in cancer patients is concerning due to the difficulty in detecting it using accurate phenotypic techniques, its correlation with therapy failure, and its spread rapidly via transferable plasmids [70].

Our findings showed that Carbapenemases-encoding genes coexisted widely (73.7%), which may be due to the ease with which these genes can be transferred between various healthcare facilities since they are carried on mobile genetic elements [71]. In 32 isolates (35.2%), both bla*NDM* and bla*VIM* genes were detected. Compared to the previous study from Egypt by **Kamel et al.** this percentage is higher[48] and **Khalil et al.** [72] in which such association was found in only 1 (3%) and 4 (8.7%) isolates, respectively. Whilst, further recent studies reported higher percentages ranging between 69% [73] and 100% [52].

In contrast, the dual coexistence of bla*NDM* and bla*KPC* was found in 11 (12.1%) of the isolates, which is close to the findings of **El-Kholy et al.** (10%)[74] but is substantially more than the results obtained by **Ragheb et al.** (5%)[73], and **Khalil et al.** (8.7%)[72]. Furthermore, bla*VIM* and bla*KPC* were found in 14(15.4%) of the isolates, which is consistent with prior studies with comparable frequencies[72, 73].

To determine the precise types of carbapenemases that are present in an isolate, it is preferable to conduct the mCIM, eCIM, and combined disc tests concurrently because they are complementing phenotypic assays. The combined disc test, compared to the mCIM test, was superior owing to higher sensitivity. Overall, for differentiating between the various carbapenemase types, the genotypic technique is the ideal and most accurate method [75].

Ethical approval

The study protocol was reviewed and approved by the Committee of Medical Ethics of the Faculty of Medicine, Assiut University, Egypt (IRB NO:17101590).

Informed consent

Informed and written consent was obtained from all patients after explaining the study's purpose.

Conflicts of interest

Not declared.

Funding

None.

References

1-Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 2022; 399(10325): 629-655.

2-Tseng Wen-Pin, Chen Yee-Chun, Chen Shang-Yu, Chen Shey-Ying, Chang Shan-Chwen. Risk for subsequent infection and mortality after hospitalization among patients with multidrug-resistant gram-negative bacteria colonization or infection. Antimicrobial Resistance & Infection Control 2018; 7:1-12.

3- **Masse J, Elkalioubie A, Blazejewski C, Ledoux G, Wallet F, Poissy J, et al.** Colonization pressure as a risk factor of ICU-acquired multidrug resistant bacteria: a prospective observational study. European Journal of Clinical Microbiology & Infectious Diseases 2017; 36:797-805.

4- **Elgendy SG, Abdel Hameed MR, El-Mokhtar MA.** Tigecycline resistance among Klebsiella pneumoniae isolated from febrile neutropenic patients. J Med Microbiol 2018; 67(7): 972-975.

5- **Numico G, Zanelli C, Ippoliti R, Rossi M , Traverso E , Antonuzzo A ,et al .** The hospital care of patients with cancer: a retrospective analysis of the characteristics of their hospital stay in comparison with other medical conditions. European Journal of Cancer 2020; 139: 99-106.

6- **Nesher L, Rolston Kenneth VI.** The current spectrum of infection in cancer patients with chemotherapy related neutropenia. Infection 2014; 42: 5-13.

7- **Zimmer A J, Stohs E, Meza J, Arnold C, Baddley J W, Chandrasekar P, et al.** Bloodstream infections in hematologic malignancy patients with fever and neutropenia: are empirical antibiotic therapies in the United States still effective? in Open Forum Infectious Diseases 2022;9(7): ofac240

8- **Cattaneo C, Di Blasi R, Skert C, Candoni A, Martino B, Di Renzo N, et al.** Bloodstream infections in haematological cancer patients colonized by multidrug-resistant bacteria. Annals of hematology 2018; 97:1717-1726.

9- **Nordmann P, Poirel L**. Epidemiology, and diagnostics of carbapenem resistance in gram-negative bacteria. Clinical Infectious Diseases 2019; 69(Supplement_7): S521-S528.

10- **Logan LK, Weinstein RA.** The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. The Journal of infectious diseases 2017;215(suppl_1): S28-S36.

11- **Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al.** Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development 2017.

12- **Mahamat O Ouchar, Lounnas M, Hide M, Tidjani A, Benavides J, Diack A, et al.** Spread of NDM-5 and OXA-181 carbapenemase-producing Escherichia coli in Chad. Antimicrobial Agents and Chemotherapy 2019; 63(11): e00646-19.

13- **Wolter DJ, Acquazzino D, Goering RV, Sammut P, Khalaf N, Hanson ND.** Emergence de la Resistance aux Carbapenemes chez Pseudomonas aeruginosa 2018.

14- **Jesudason MV, Kandathil AJ, Veeraraghavan B.** Comparison of two methods to detect carbapenemase & metallo-beta-lactamase production in clinical isolates. Indian Journal of Medical Research 2005;121(6): 780.

15- **Queenan AM, Bush K.** Carbapenemases: the versatile β-lactamases. Clinical microbiology reviews 2007; 20(3): 440-458.

16- **Mathers AJ, Cox HL, Kitchel B, Bonatti H, Brassinga AKC, Carroll J, et al.** Molecular dissection of an outbreak of carbapenem-resistant Enterobacteriaceae reveals intergenus KPC carbapenemase transmission through a promiscuous plasmid. MBio 2011; 2(6): e00204-11.

17- **Perez F, Van Duin D.** Carbapenem-resistant Enterobacteriaceae: a menace to our most vulnerable patients. Cleveland Clinic Journal of Medicine 2013;80(4): 225.

18- **Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, et al.** A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP-and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. Journal of Antimicrobial Chemotherapy 2018; 73(4): 909-915.

19- **Abdel-Baky RM, Ali MA, Abuo-Rahma GEAA, AbdelAziz N.** Inhibition of urease enzyme production and some other virulence factors expression in Proteus mirabilis by N-acetyl cysteine and dipropyl disulfide. Advances in Microbiology, Infectious Diseases and Public Health 2017; 7:99-113.

20- **Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al.** Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection 2012;18(3): 268-281.

21- **Wayne, PA.** Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing,30th ed. CLSI supplement M100 Clinical and Laboratory Standards Institute 2020.

22- **Clinical Laboratory Standards Institute (CLSI).** Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9. CLSI 2018; 32:18.

23- **Sfeir MM, Hayden JA, Fauntleroy KA, Mazur C, Johnson JK, Simner PJ, et al.** EDTA-modified carbapenem inactivation method: a phenotypic method for detecting metallo-β-lactamase-producing Enterobacteriaceae. Journal of clinical microbiology 2019; 57(5): e01757-18.

24- **Tsakris A, Poulou A, Pournaras S, Voulgari E, Vrioni G, Themeli-Digalaki K, et al .** A simple phenotypic method for the differentiation of metallo-β-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. Journal of antimicrobial chemotherapy 2010; 65(8):1664-1671.

25- **Junior JCR, Tamanini R, Soares BF, Oliveira AM, Silva F de Godoi, Silva FF,et al.** Efficiency of boiling and four other methods for genomic DNA extraction of deteriorating spore-forming bacteria from milk. Semina: Ciências Agrárias 2016; 37(5): 3069-3078.

26- **Nordmann P, Naas T, Poirel L.** Global spread of carbapenemase-producing Enterobacteriaceae. Emerging infectious diseases 2011; 17(10): 1791.

27- **Österblad M, Kirveskari J, Hakanen AJ, Tissari P, Vaara M, Jalavaet J.** Carbapenemase-producing Enterobacteriaceae in Finland: the first years (2008–11). Journal of antimicrobial chemotherapy 2012; 67(12): 2860-2864.

28- **Corbellini S, Caccuri F, Gelmi M, De Francesco MA, Fiorentini S, Caruso A, et al.** Emergence of carbapenem-resistant Klebsiella Pneumoniae strains producing KPC-3 in Brescia Hospital, Italy. The New Microbiologica 2014; 37(2):177-183.

29- **Bhat S, Muthunatarajan S, Mulki SS, Bhat KA, Kotian KH.** Bacterial infection among cancer patients: analysis of isolates and antibiotic sensitivity pattern. International journal of microbiology 2021.

30- **Perdikouri EIA, Arvaniti K, Lathyris D, Apostolidou KF, Siskou E, Haidich AB, et al.** Infections due to multidrug-resistant bacteria in oncological patients: insights from a five-year epidemiological and clinical analysis. Microorganisms 2019; 7(9): 277.

31- **Lakshmaiah KC, Malabagi AS, Govindbabu, Shetty R, Sinha M, Jayashree RS.** Febrile neutropenia in hematological malignancies: clinical and microbiological profile and outcome in high risk patients. Journal of laboratory physicians 2015; 7(02): 116-120.

32- **Tamai Y, Imataki O, Kawakami K.** Fever profile of febrile neutropenia in patients treated with cancer chemotherapy for hematological malignancies. Gan to Kagaku ryoho. Cancer & Chemotherapy 2010; 37(5): 859-862.

33- **Gençer S, Salepçi T, Özer S.** Evaluation of infectious etiology and prognostic risk factors of febrile episodes in neutropenic cancer patients. Journal of Infection 2003;47(1): 65-72.

34- **El-Mahallawy H, Sidhom I, El-Din NH, Zamzam M, El-Lamie MM.** Clinical and microbiologic determinants of serious bloodstream infections in Egyptian pediatric cancer patients: a one-year study. International journal of infectious diseases 2005; 9(1): 43-51.

35- **Soroush J, Razavi SM, Nojoomi M.** Comparing Two Empiric Antibiotic Regimens in Treatment of Febrile Neutropenic Cancer Patients. Razi Journal of Medical Sciences 2003, 10(36): 547-552.

36- **Kuo FC, Wang SM, Shen CF, Ma YJ, Ho TS, Chen JS, et al.** Bloodstream infections in pediatric patients with acute leukemia: Emphasis on gram-negative bacteria infections. Journal of Microbiology, Immunology, and Infection 2017;50(4): 507-513.

37- **Yao JF, Li N, Jiang J.** Clinical characteristics of bloodstream infections in pediatric acute leukemia: a single-center experience with 231 patients. Chinese medical journal 2016;130(17): 2076-2081.

38- **Babypadmini S, Appalaraju B.** Extended spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae-prevalence and susceptibility pattern in a tertiary care hospital. Indian Journal of medical microbiology 2004; 22(3):172-174.

39- **Azene MK, Beyene BA.** Bacteriology and antibiogram of pathogens from wound infections at Dessie Laboratory, North East Ethiopia. Tanzania Journal of health research 2011; 13(4).

40- **Hailu D, Derbie A, Mekonnen D, Zenebe Y, Adem Y, Worku S, et al.** Drug resistance patterns of bacterial isolates from infected wounds at Bahir Dar Regional Health Research Laboratory center, Northwest Ethiopia. Ethiopian Journal of Health Development 2016; 30(3): 112-117.

41- **Beyene D, Bitew A, Fantew S, Mihret A, Evans M.** Multidrug-resistant profile and prevalence of extended spectrum β-lactamase and carbapenemase production

in fermentative Gram-negative bacilli recovered from patients and specimens referred to National Reference Laboratory, Addis Ababa, Ethiopia. PloS one 2019; 14(9): e0222911.

42- **Reinheimer C, Kempf VAJ, Göttig S, Hogardt M, Wichelhaus TA, O'Rourke F, et al**. Multidrug-resistant organisms detected in refugee patients admitted to a University Hospital, Germany June–December 2015. Eurosurveillance 2016; 21(2): 30110.

43- **Tenenbaum T, Becker KP, Lange B, Martin A, Schäfer P, Weichert S, et al.** Prevalence of multidrug-resistant organisms in hospitalized pediatric refugees in a University Children's Hospital in Germany 2015–2016. infection control & hospital epidemiology 2016; 37(11): 1310-1314.

44- **Cornejo-Juárez P, Vilar-Compte D, Pérez-Jiménez C, Ñamendys-Silva SA, Sandoval-Hernández S, Volkow-Fernández P.** The impact of hospital-acquired infections with multidrug-resistant bacteria in an oncology intensive care unit. International Journal of Infectious Diseases 2015; 31:31-34.

45- **Prata-Rocha ML, Moreira MR, Gontijo Filho PP, Melo GB.** Acinetobacter baumannii: Global Evolution of Carbapenem-Resistant and Genotyping Methods. Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education 2013;1:197-203.

46- **Hassler A, Bochennek K, Gilfert J, Perner C, Schöning S, Creutzig U, et al.** Infectious complications in children with acute myeloid leukemia: decreased mortality in multicenter trial AML-BFM 2004. Blood cancer journal 2016; 6(1): e382-e382.

47- **Lee YH, Cho B, Bae IK, Chang CL, Jeong SH.** Klebsiella pneumoniae strains carrying the chromosomal SHV-11 β-lactamase gene produce the plasmid-mediated SHV-12 extended-spectrum β-lactamase more frequently than those carrying the chromosomal SHV-1 β-lactamase gene. Journal of Antimicrobial Chemotherapy 2006; 57(6): 1259-1261.

48- **Kamel NA, El-Tayeb WN, El-Ansary MR, Mansour MT, Aboshanab KM.** Phenotypic screening and molecular characterization of carbapenemase-producing Gram-negative bacilli recovered from febrile neutropenic pediatric cancer patients in Egypt. PloS one 2018; 13(8): e0202119.

49- **Tawfick MM, Alshareef WA, Bendary HA, Elmahalawy H, Abdulall AK.** The emergence of carbapenemase bla NDM genotype among carbapenem-resistant Enterobacteriaceae isolates from Egyptian cancer patients. European Journal of Clinical Microbiology & Infectious Diseases 2020; 39: 1251-1259.

50- **Osama D, El-Mahallawy H, Mansour MT, Hashem A, Attia AS.** Molecular characterization of carbapenemase-producing Klebsiella pneumoniae isolated from Egyptian pediatric cancer patients including a strain with a rare gene-combination of β-lactamases. Infection and Drug Resistance 2021; 335-348.

51- **Kotb S, Lyman M, Ismail G, Abd El Fattah M, Girgis SA, Etman A, et al.** Epidemiology of carbapenem-resistant Enterobacteriaceae in Egyptian intensive

care units using National Healthcare–associated Infections Surveillance Data, 2011–2017. Antimicrobial Resistance & Infection Control 2020;9(1): 1-9.

52- **Hassuna NA, AbdelAziz RA, Zakaria A, Abdelhakeem M.** Extensively-drug resistant Klebsiella pneumoniae recovered from neonatal sepsis cases from a major NICU in Egypt. Frontiers in microbiology 2020; 11: 1375.

53- **Juhász E, Iván M, Pongrácz J, Kristóf K.** Uncommon non-fermenting Gram-negative rods as pathogens of lower respiratory tract infection. Orvosi hetilap 2018; 159(1): 23-30.

54- **Bialvaei AZ, Kouhsari E, Salehi-Abargouei A, Amirmozafari N, Ramazanzadeh R, Ghadimi-Daresajini A, et al.** Epidemiology of multidrug-resistant Acinetobacter baumannii strains in Iran: a systematic review and meta-analysis. Journal of Chemotherapy 2017.;29(6): 327-337.

55- **Tijet N, Patel SN, Melano RG.** Detection of carbapenemase activity in Enterobacteriaceae: comparison of the carbapenem inactivation method versus the Carba NP test. Journal of Antimicrobial Chemotherapy 2016; 71(1): 274-276.

56- **Zhou M, Wang D, Kudinha T, Yang Q, Yu S, Xu YC.** Comparative evaluation of four phenotypic methods for detection of class A and B carbapenemase-producing Enterobacteriaceae in China. Journal of clinical microbiology 2018; 56(8): e00395-18.

57- **Tängdén T, Giske CG.** Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment, and infection control. Journal of internal medicine 2015; 277(5): 501-512.

58- **Bansal M, Vyas N, Sharma B.** Differentiation of carbapenemase producing Enterobacteriaceae by triple disc test. Indian J Basic Appl Med Res 2013; 3(1): 314-20.

59- **Baraniak A, Izdebski R, Fiett J, Sadowy E, Adler A, Kazma M, et al.** Comparative population analysis of Klebsiella pneumoniae strains with extended-spectrum β-lactamases colonizing patients in rehabilitation centers in four countries. Antimicrobial agents and chemotherapy 2013,57(4): 1992-1997.

60- **Nordmann P, Cuzon G, Naas T.** The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. The Lancet infectious diseases 2009; 9(4): 228-236.

61- **Chmelnitsky I, Navon-Venezia S, Strahilevitz J, Carmeli Y.** Plasmid-mediated qnrB2 and carbapenemase gene bla KPC-2 carried on the same plasmid in carbapenem-resistant ciprofloxacin-susceptible Enterobacter cloacae isolates. Antimicrobial Agents and Chemotherapy 2008; 52(8): 2962-2965.

62- **Endimiani A, Carias LL, Hujer AM, Bethel CR, Hujer KM, Perez F,et al.** Presence of plasmid-mediated quinolone resistance in Klebsiella pneumoniae isolates possessing bla KPC in the United States. Antimicrobial agents and chemotherapy 2008; 52(7): 2680-2682.

63- **Tsakris A, Kristo I, Poulou A, Markou F, Ikonomidis A, Pournaras S.** First occurrence of KPC-2-possessing Klebsiella pneumoniae in a Greek hospital

and recommendation for detection with boronic acid disc tests. Journal of antimicrobial chemotherapy 2008; 62(6):1257-1260.

64- **Rudresh SM, Ravi GS, Sunitha L, Hajira SN, Kalaiarasan E, Harish BN.** Simple, rapid, and cost-effective modified Carba NP test for carbapenemase detection among Gram-negative bacteria. Journal of laboratory physicians 2017; 9(04): 303-307.

65- **Erfan D, Ibrahim W.** Evaluation of direct Carba-NP (CNPt) for screening of carbaenemases production in Gram negative bacilli. Microbes and Infectious Diseases 2022; 3(2): 378-386.

66- **Elnagar RM**. Evaluating the performance of different detection methods of Carbapenemase producing Gram-negative bacilli isolated from surgical site infections. Novel Research in Microbiology Journal 2021; 5(2): 1194-1213.

67- **Carattoli A.** Plasmids and the spread of resistance. IJMM 303 2013: 298–304.

68- **Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al.** Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet infectious diseases 2010; 10(9):597-602.

69- **Nordmann P, Poirel L, Toleman MA, Walsh TR.** Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for the treatment of infections caused by Gram-negative bacteria? Journal of antimicrobial chemotherapy 2011; 66(4): 689-692.

70- **Bakthavatchalam YD, Anandan S, Veeraraghavan B.** Laboratory detection and clinical implication of oxacillinase-48 like carbapenemase: the hidden threat. Journal of global infectious diseases 2016; 8(1): 41.

71- **El-Badawy MF, El-Far SW, Althobaiti SS, Abou-Elazm FI, Shohayeb MM.** The First Egyptian report showing the co-existence of bla NDM-25, bla OXA-23, bla OXA-181, and bla GES-1 among carbapenem-resistant K. pneumoniae clinical isolates genotyped by BOX-PCR. Infection and Drug Resistance 2020; 1237-1250.

72- **Khalil MAF, Elgaml A, El-Mowafy M.** Emergence of multidrug-resistant New Delhi metallo-β-lactamase-1-producing Klebsiella pneumoniae in Egypt. Microbial Drug Resistance 2017; 23(4): 480-487.

73- **Ragheb SM, Tawfick MM, El-Kholy AA, Abdulall AK**. Phenotypic and genotypic features of klebsiella pneumoniae harboring carbapenemases in Egypt: OXA-48-like carbapenemases as an investigated model. Antibiotics 2020; 9(12): 852.

74- **El-Kholy AA, Elanany MG, Sherif MM, Gad MA.** High prevalence of VIM, KPC, and NDM expression among surgical site infection pathogens in patients having emergency surgery. Surgical infections 2018; 19(6): 629-633.

75- **Chavda KD, Satlin MJ, Chen L, Manca C, Jenkins SG, Walsh TJ, et al.** Evaluation of a multiplex PCR assay to rapidly detect Enterobacteriaceae with a broad range of β-lactamases directly from perianal swabs. Antimicrobial agents and chemotherapy 2016; 60(11): 6957-6961.