ORIGINAL ARTICLE

Hospital-Acquired Infection by Carbapenem-Resistant *Acinetobacter* Species in ICUs in Assiut, Egypt

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ABSTRACT

Background: *The genus Acinetobacter is one of the main fears of hospital-acquired infection (HAI) wide-reaching mostly in ICUs.* ***Objectives:*** *This study aimed to identify the incidence of carbapenem-resistant Acinetobacter (CRA) HAI in Assiut University Hospitals ICU and determine their antimicrobial susceptibility profile.* ***Methodology:*** *The study included 1204 clinical samples from 928 patients with HAI and 625 environmental samples. Carbapenemase production was detected phenotypically by MIC determination using imipenem, meropenem and doripenem E-test, modified Hodge test, combined disc test and double disc synergy test (DDST). OXA genes (bla_{OXA-51}, bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58}) and MβL genes (bla_{SIM-1}, bla_{VIM}, and bla_{IMP-1}) were investigated genotypically by PCR.* ***Results:*** *Out of total 60 Acinetobacter isolates, 55 (91.7%) were extensively drug-resistant (XDR) and 5 (8.3%) were multi-drug resistant (MDR). 93.3% of Acinetobacter isolates were resistant to imipenem and meropenem, and 90% were resistant to doripenem. The prevalence of bla genes in isolates were bla_{OXA-51} (93.3%), bla_{OXA-23} (68.3%), bla_{OXA-24} (23.3%), bla_{OXA-58} (10%), bla_{SIM-1} (46.7%), and bla_{VIM} (8.3%). bla_{IMP-1} was not detected in any isolate.* ***Conclusion:*** *CRA infections were considerable in our ICUs. The high prevalence of XDR Acinetobacter spp. observed in our study was very alarming and requires critical intervention.*

INTRODUCTION

The genus *Acinetobacter* is commonly dispersed in nature. It has been related with hospital-acquired infection (HAI), especially in critically sick patients and ICUs[1]. *A. baumannii* is the most clinically relevant species and has arisen as a main problem in health institutions due to its high propensity to gain resistance to antibiotics[2]. It has blowout worldwide becoming one of the frequent and main causes of HAI[3]. Corresponding to increased incidence of *A. baumannii* infections, various and contesting resistance mechanisms are identified in this pathogen via a broad display of antibiotic-hydrolyzing enzymes, impermeability, efflux pump changes, and antibiotic target mutations, along with main classes of antibiotics becoming less effective[4]. Antimicrobial resistance possessed by *Acinetobacter* strains is mediated by hasty gene mutations or transmission of exogenous resistance genes by plasmids, transposons, or insertion sequences. *A. baumannii* has the tendency for gaining multiple resistance genes with phenotypic expression of multidrug-resistant (MDR) features. Extensively drug-resistant (XDR) strains display resistance to all but two antimicrobial drug classes[5-7]. The production of β-lactamases is the main mechanism of *Acinetobacter* spp. resistance to β-lactam antibiotics, they are capable to hydrolyze carbapenems in consort with other β-lactams. Carbapenemases are precise β-lactamases with the capability to hydrolyze carbapenems[8]. The crucial role of *Acinetobacter* spp. resistance to carbapenems, is arbitrated chiefly by oxacillinases (OXA-class D) and, less commonly, by MβLs (MβL-class B). Class D carbapenemases are the major carbapenemases in *A. baumannii*[9].

Carbapenem-resistant *Acinetobacter* (CRA) which are known to be sensitive to tigecycline and polymyxins, are increasing worldwide, rendering *Acinetobacter* infection challenging to cure. Unfortunately, emerging resistance has evolved limiting treatment options. The objectives of this study were to identify the prevalence of HAI caused by CRA in different ICUs of Assiut University Hospitals, determine their antimicrobial susceptibility profile and investigate the distribution of OXA and MβL genes of carbapenem resistance among *Acinetobacter* isolates.

METHODOLOGY

Specimen collection:

This study was conducted on 1204 clinical samples obtained from 928 patients with HAI who were hospitalized in different ICUs of Assiut University Hospitals. Samples included endotracheal aspirates (n= 546), sputum (n= 218), blood (n= 217), urine (n= 161), throat swabs (n= 35), wound swabs (n= 24), ear swab

(n= 1), rectal swab (n= 1) and bed sores (n= 1). The study also included 625 environmental swabs that were collected from surfaces, walls, furniture, curtains, ventilators, monitor touch keys, beds, and trolleys of ICUs' wards of Assiut University Hospitals.

Identification of the recovered bacterial isolates:

All samples were transported to Infection Control Laboratory and inoculated on regular bacteriological media, including blood agar, MacConkey agar and Herellea agar (HiMedia, India) for isolation of *Acinetobacter* strains. Suspected colonies were identified conventionally using Gram stain and standard biochemical reactions. The isolates were identified up to the species level using Vitek 2 system (BioMerieux, France).

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was done by the Kirby-Bauer disc diffusion method according to CLSI guidelines[10]. Antibiotics tested were Piperacillin (100µg), Piperacillin/Tazobactam (100/10µg), Cefoperazone (75µg), Ceftazidime (30µg), Cefepime (30µg), Aztreonam (30µg), Amikacin (30µg), Gentamicin (10µg), Levofloxacin (5µg), Ciprofloxacin (5µg), Imipenem (10µg), Meropenem (10µg), Tigecycline (15µg), Colistin (10µg) and Polymyxin B (10µg) (Hi-Media, India). *E. coli* ATCC25922 was used as control strain.

Phenotypic detection of carbapenemase:

All *Acinetobacter* isolates were tested for accurate determination of their MIC by imipenem-E test, meropenem-E test, and doripenem-E test (bioMérieux SA, Marcy l'Etoile, France) using CLSI breakpoints. Modified Hodge test (MHT) using imipenem and meropenem discs (10 µg) (Hi-Media, India) was used according to CLSI recommendations to detect carbapenemase production[10].

Detection of MβLs was done by combined disc test (CDT)[11] and double disc synergy test (DDST)[12] using imipenem and meropenem discs (10 µg) and they were placed 13 mm (center to center) from EDTA disc as a modification.

Molecular detection of carbapenemase genes in *Acinetobacter* isolates:

Genomic DNAs of all 60 *Acinetobacter* strains were extracted by boiling method[13]. All strains were tested for class B $bla_{\text{IMP-1}}$, bla_{VIM}, $bla_{\text{SIM-1}}$ genes and class D $bla_{\text{OXA-51}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-23}}$, and $bla_{\text{OXA-58}}$ genes by PCR using the proper primers[14-16] (table 1) and Taq DNA polymerase kit (Promega, USA) in a thermal cycler (BioRadT100, USA).

Table 1: Sequence of primers used in PCR amplification of genes encoding MβLs and Oxacillinases in *Acinetobacter* isolates

Class	Genes	Primers Sequence (5'-3')	Size(bp)
Class B	$bla_{\text{IMP-1}}$	F (-CAT GGT TTG GTG GTT CTT GT-) R (-ATA ATT TGG CGG ACT TTG GC-)	488
	bla_{VIM}	F (-GTT TGG TCG CAT ATC GCA AC-) R (-AAT GCG CAG CAC CAG GAT AG)	382
	$bla_{\text{SIM-1}}$	F (-GTA CAA GGG ATT CGG CAT CG-) R (-TGG CCT GTT CCC ATG TG AG-)	569
Class D	$bla_{\text{OXA-51}}$	F (-TAA TGC TTT GAT CGG CCT TG-) R (-TGG ATT GCA CTT CAT CTT GG-)	353
	$bla_{\text{OXA-23}}$	F (-GAT CGG ATT GGA GAA CCA GA-) R (-ATT TCT GAC CGC ATT TCC AT-)	501
	$bla_{\text{OXA-24}}$	F (-GGT TAG TTG GCC CCC TTA AA-) R (-AGT TGA GCG AAA AGG GGA TT)	246
	$bla_{\text{OXA-58}}$	F (-AAG TAT TGG GGC TTG TGC TG-) R (-CCC CTC TGC GCT CTA CAT AC-)	599

RESULTS

Identification of the bacterial isolates:

The present work is a hospital based descriptive study conducted on 1204 clinical samples collected from 928 patients with HAI who were admitted to different ICUs at Assiut University Hospitals. Of 2340 isolated pathogens, 1551 (66.3%) were Gram-negative bacilli, 538 (23%) were *Staphylococci* spp., and 251 (10.7%) were *Candida* spp. The frequency of *Acinetobacter* isolates among 928 patients with HAI was 55 (5.9%). They included 51 (92.7%) *A. baumannii* and 4 (7.3%) *ABC complex*. The highest percentage of *Acinetobacter* isolates was observed in endotracheal aspirates (54.5%), followed by sputum (25.5%), blood (7.3%), urine (5.5%), wound swabs (5.5%), and throat swabs (1.8%). No *Acinetobacter* strains were isolated from ear swabs, bed sores or rectal swabs.

Acinetobacter isolates were mostly isolated from chest ICU (56.36%), followed by neurology ICU (16.36%), trauma ICU (12.73%), hematology, pediatrics, and

gynecology ICUs (3.64%) each and lastly general and postoperative ICUs (1.82%) each as shown in table (2).

Table 2: Distribution of *Acinetobacter* isolates among GNB of clinical samples collected from different ICUs.

ICU	No. of samples collected	No. of isolated GNB	*Acinetobacter* isolates		
			No.	% From total number of GNB isolated from each ICU	% From total No. of *Acinetobacter* isolates (n=55)
Chest ICU	497	723	31	4.29%	56.36%
Trauma ICU	230	215	7	3.26%	12.73%
Neurology ICU	180	325	9	2.77%	16.36%
Hematology ICU	147	130	2	1.54%	3.64%
Postoperative ICU	42	44	1	2.27%	1.82%
Tropical ICU	28	26	0	0%	0%
Pediatrics ICU	21	27	2	7.41%	3.64%
Coronary ICU	15	10	0	0%	0%
Gynecology ICU	5	6	2	33.3%	3.64%
Internal medicine ICU	4	3	0	0%	0%
Total	**1204**	**1551**	**55**	**---**	**100%**

The study also included 625 environmental samples and contamination was confirmed in 443 samples representing (70.88%). 5 *Acinetobacter* strains were isolated representing (0.8%). They were identified as *A. baumannii*, and the highest percentage was isolated from neurology ICU.

Antimicrobial susceptibility testing:

The highest rates of resistance of *Acinetobacter* strains in clinical samples were against piperacillin, ceftazidime, cefepime and aztreonam (100%). Also, they were highly resistant to cefoperazone and piperacillin/tazobactam (96.36% for each), ciprofloxacin and levofloxacin (94.55% for each), gentamicin (92.73%), meropenem (90.9% for each), amikacin (89.09%), and imipenem (87.27%). While the highest rates of susceptibility were to polymyxin B (100%) followed by tigecycline (92.73%) as demonstrated in table (3). In environmental samples (n= 5), all isolates were resistant to all antimicrobial drugs (100%) except to polymyxin, tigecycline and colistin (0%).

Out of total 60 *Acinetobacter* isolates, 55 (91.7%) isolates were extensively drug-resistant (XDR), and 5 (8.3%) isolates were multi-drug resistant (MDR) according to the conventional disc diffusion method.

Table 3: Resistance pattern of *Acinetobacter* isolates in clinical samples (n= 55) to various antimicrobial agents.

Antimicrobial agent	No. of resistant isolates (%)	No. of intermediate isolates (%)	No. of sensitive isolates (%)
Piperacillin	55 (100%)	0 (0%)	0 (0%)
Piperacillin/tazobactam	53 (96.36%)	2 (3.64%)	0 (0%)
Ceftazidime	55 (100%)	0 (0%)	0 (0%)
Cefepime	55 (100%)	0 (0%)	0 (0%)
Imipenem	48 (87.27%)	0 (0%)	7 (12.73%)
Meropenem	50 (90.9%)	0 (0%)	5 (9.09%)
Gentamicin	51 (92.73%)	2 (3.63%)	2 (3.63%)
Amikacin	49 (89.09%)	1 (1.82%)	5 (9.09%)
Ciprofloxacin	52 (94.55%)	0 (0%)	3 (5.45%)
Levofloxacin	52 (94.55%)	0 (0%)	3 (5.45%)
Aztreonam	55 (100%)	0 (0%)	0 (0%)
Cefoperazone	53(96.36%)	0 (0%)	2 (3.63%)
Tigecycline	2 (3.63%)	2 (3.63%)	51 (92.73%)
Colistin	9 (16.36%)	18 (32.73%)	28 (50.91%)
Polymyxin B	0 (0%)	0 (0%)	55 (100%)

Phenotypic carbapenemase detection:

All *Acinetobacter* isolates were tested for accurate determination of their MIC using the imipenem-E test, meropenem-E test, and doripenem-E test strips. Carbapenem resistance was detected in 93.3% by imipenem and meropenem -E tests and in 90% by

doripenem-E test. MHT identified carbapenem resistance in 73.3% using imipenem disc and in 55% using meropenem disc. All imipenem, meropenem and doripenem-E tests susceptible *Acinetobacter* isolates showed negative MHT results. MβL activity was identified in 75% by CDT using imipenem disc, 63.3% by CDT using meropenem disc, 56.7% by DDST using imipenem disc and 46.7% by DDST using meropenem disc (table 4).

Table 4: Phenotypic carbapenemase detection

Phenotypic test		Clinical samples (n=55)	Environmental samples (n=5)	Total (n=60)
imipenem-E test	Susceptible	4 (7.27%)	0 (0%)	4 (6.67%)
	Resistant	51 (92.73%)	5 (100%)	56 (93.3%)
meropenem-E test	Susceptible	4 (7.27%)	0 (0%)	4 (6.67%)
	Resistant	51 (92.73%)	5 (100%)	56 (93.3%)
doripenem-E test	Susceptible	6 (10.9%)	0 (0%)	6 (10%)
	Resistant	49 (89%)	5 (100%)	54 (90%)
MHT using imipenem disc	Positive	40 (72.73%)	4 (80%)	44 (73.33%)
	Negative	15 (27.27%)	1 (20%)	16 (26.67%)
MHT using meropenem disc	Positive	29 (52.73%)	4 (80%)	33 (55%)
	Negative	26 (47.27%)	1 (20%)	27 (45%)
CDT using imipenem disc	Positive	41 (74.5%)	4 (80%)	45 (75%)
	Negative	14 (25.5%)	1 (20%)	15 (25%)
CDT using meropenem disc	Positive	34 (61.82%)	4 (80%)	38 (68.3%)
	Negative	21 (38.18%)	1 (20%)	22 (36.67%)
DDST using imipenem disc	Positive	32 (58.18%)	2 (40%)	34 (56.67%)
	Negative	23 (41.82%)	3 (60%)	26 (43.33%)
DDST using meropenem disc	Positive	26 (47.27%)	2 (40%)	28 (46.67%)
	Negative	29 (52.73%)	3 (60%)	32 (53.33%)

Molecular detection of OXA and MβL encoding genes:

A total of 60 *Acinetobacter* isolates were tested for the presence of carbapenemases encoding genes (bla_{IPM-1}, bla_{VIM}, bla_{SIM-1}, bla_{OXA-51}, bla_{OXA-24}, bla_{OXA-23}, and bla_{OXA-58}) by conventional PCR.

The distribution of carbapenemases genes in 60 *Acinetobacter* isolates is displayed in figure (1). The most prevalent acquired genes were bla_{OXA-23} (68.33%) followed by bla_{SIM-1} (46.67%), bla_{OXA-24} (23.33%), bla_{OXA-58} (10%), and bla_{VIM} (8.3%). While bla_{IPM-1} was negative in all strains. All *A. baumannii* isolates exhibited bla_{OXA-51} gene while none of the 4 isolates which belong to ACB complex according to VITEK 2 system identification contained this gene. Moreover, two carbapenem susceptible strains that showed negative results in all phenotypic tests were harboring hidden MβL genes.

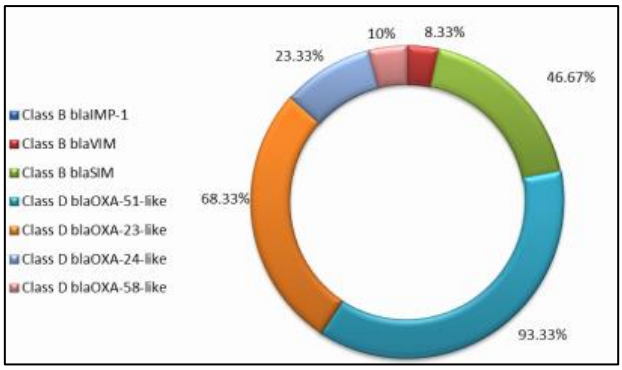

Fig. 1: Distribution of carbapenemases genes in 60 *Acinetobacter* isolates.

Out of 56 carbapenem-resistant strains, 46 (82.1%) *Acinetobacter* isolates harbored more than one of carbapenemase genes and the most combination forms were (bla_{OXA-51} + bla_{OXA-23}), (bla_{SIM-1} + bla_{OXA-51} + bla_{OXA-23}) and (bla_{SIM-1} + bla_{OXA-51} + bla_{OXA-24} + bla_{OXA-23}) with percentages of 18.3%, 16.7% and 11.7% respectively.

DISCUSSION

Acinetobacter spp. mostly infect patients in ICUs who are critically ill. Lately, *Acinetobacter* has gotten more consideration owing to its great propensity to gain resistance to several antibiotics including carbapenems and the development of CRA isolates has come to be a serious worldwide concern. The present study aims to determine the prevalence of HAI caused by CRA in different ICUs of Assiut University Hospitals, their antimicrobial susceptibility profile, and the distribution of OXA and MβL genes of carbapenem-resistance in *Acinetobacter* isolates.

In this study, the incidence of *Acinetobacter* HAI in Assiut University Hospitals ICUs was 5.9%. This was in accordance with the results of Mancini et al.[17] who declared that the incidence of *Acinetobacter* HAI in an Italian tertiary care hospital was 3.4%. However, our results are higher than those observed by Nageeb et al.[18], who recovered 10 (2.9%) *Acinetobacter* isolates from 350 ICU patients. On the other hand, our results were less than those reported by Shalaby et al.[19] and Adel and Mohamed[20] in Egypt, who isolated 20 and 40 *Acinetobacter* strains from 129 (15.5%) and 175 (22.9%) patients, respectively. Moreover, a study conducted by Aljindan et al.[21] in Saudi Arabia reported that the incidence of *Acinetobacter* infections was 47/565 (8.3%). This variation could be attributable to the different study population and different applied infection control policies.

In the current study, the highest number of *Acinetobacter* strains were recovered from chest ICU with a percentage of 54.36%, which is not in concordance with the findings of Shalaby et al., who reported that 37% of collected *Acinetobacter* strains were isolated from pediatric ICU[19]. Despite of this difference, both studies revealed that respiratory tract was the most common site of *Acinetobacter* infections in ICUs.

In the present study, out of 625 different environmental samples from different ICUs, five *Acinetobacter* isolates were identified (0.8%). This finding agrees to some extent with the results of Kirkgöz and Zer, who recovered eight *Acinetobacter* isolates from 400 environmental samples (2%)[22]. On the contrary, our results are less than those reported by Shalaby et al. (18.2%)[19], due to the difference in samples size and applied infection control policies in each healthcare setting even in the same country. *Acinetobacter* has multiple virulence factors, including capsule, porins, cell wall lipopolysaccharide, enzymes, biofilm production, motility, and iron-acquisition systems. All these factors help the organism to resist stressful environmental conditions in hospitals and overcome infection control measures.

In our study, 92.7% and 7.3% of clinical *Acinetobacter* isolates were identified as *A. baumannii* and *ACB complex*, respectively. Our findings concur to some extent with those reported by Gheorghe et al.[23], who identified 89% (49/55), 7.3% (4/55) and 3.6% (2/55) of collected *Acinetobacter* strains as *A. baumannii*, *A. haemolyticus* and *A. lwoffii*, respectively. Furthermore, all our environmental samples were identified as *A. baumannii*. which is in accordance with the results of Uwingabiye et al.[24].

Our study revealed that the highest rates of resistance were against piperacillin, piperacillin/ tazobactam, ceftazidime, cefepime and aztreonam (100%). Also, *Acinetobacter* isolates were highly resistant to cefoperazone and gentamicin (96.36% for each), ciprofloxacin and levofloxacin (94.55% for each), amikacin and meropenem (90.9% for each) and imipenem (87.27%). The resistance pattern of the isolates was moderate against colistin (49.09%) and low against tigecycline (7.26%). The most effective drug against *Acinetobacter* isolates was polymyxin B with 0% resistance. The high resistance rates might be associated with antibiotic abuse and prolonged ICU stays. Our results contradict those of Tafreshi et al. who reported that all isolated *A. baumannii* strains were sensitive to colistin[25]. However, our results corroborate to high extent with other studies, which stated that the most effective antibiotics for *Acinetobacter* strains were polymyxin B with resistance rate of (0%)[26,27]. A published study in Iran reported that the lowest resistance rate of *Acinetobacter* isolates was observed against polymyxin B but with a percentage of (19.35%)[28]. All these findings support the evidence that polymyxins (B or E) have increasingly become the last available therapeutic option for XDR *Acinetobacter* infections in critically ill patients despite their neurotoxicity and nephrotoxicity side effects.

Referring to tigecycline resistance, our finding was less than those of Aljindan et al.[21] who reported that tigecycline resistance of *Acinetobacter* isolates was (17.72%).

The percentage of colistin resistance in this study was less than what reported in another study in USA[29], which declared that colistin resistance of *A. baumannii* isolates was (100%), conversely it was higher than that of Leite et al., who reported that colistin resistance in Brazil was (35%)[30].

In the current study 91.7% of *Acinetobacter* isolates were XDR. Additionally, López-Durán et al.[5] and Alnimr et al.[31] reported that 100% of *Acinetobacter* isolates were XDR. However, these results were higher than those published by Ziólkowski et al.[32] from Poland, who reported that 76.5% of *Acinetobacter* isolates were XDR. The previous findings revealed that XDR *Acinetobacter* spp. have become a clinical threat in many hospital ICUs in different geographical regions. This is mainly attributed to the extensive and misuse of

antibiotics from different classes particularly carbapenems in ICUs.

It was noticed that doripenem-E test had more specific results compared to imipenem and meropenem-E tests. Furthermore, imipenem-MHT had more sensitive and accurate results compared to meropenem-MHT. However, it may not be a useful screening test for carbapenemases detection as it missed some MβL producing isolates.

Moreover, meropenem-DDST had higher accurate and specific results compared to CDT results. It can be used as a convenient screening method For MβL detection in microbiology laboratories. As it is simple, rapid, and cost-effective. However, the difficulty in discrimination between synergism and overlapping of inhibition zones is the only limitation of this method.

Oxacillinase encoding gene *bla*$_{\text{OXA-23}}$ was detected in the vast majority of *Acinetobacter* isolates 68.3% (41/60). Our findings are consistent with those of Amr and Abdel Razek[33], who detected the *bla*$_{\text{OXA-23}}$ gene in 69.7% of carbapenem resistant *A. baumannii* (CRAB) strains. However, higher rates were noted in other studies. A study conducted in South Africa reported the *bla*$_{\text{OXA-23}}$ gene in 99% of their study samples[34]. Another study in Iraq, also showed a wide distribution of *bla*$_{\text{OXA-23}}$ gene between isolates (100%)[35].

It was found that 23.3% (14/60) and 10% (6/60) of *Acinetobacter* isolates harbor *bla*$_{\text{OXA-24}}$ gene and *bla*$_{\text{OXA-58}}$ gene, respectively. Concurring with our findings, Shoja et al.[27] declared that 20 % of CRA were positive for *bla*$_{\text{OXA-24}}$ gene. However, Ghaith et al.[36], reported that neither *bla*$_{\text{OXA-24}}$ gene nor *bla*$_{\text{OXA-58}}$ gene were detected in *Acinetobacter* strains, which contradict our findings.

In the current study oxacillinase encoding gene *bla*$_{\text{OXA-51}}$ was exhibited by all *A. baumanii* isolates. Similarly, a study published by Chessab and Shubbar[35] showed that *bla*$_{\text{OXA-51}}$ gene was possessed by all *A. baumanii* isolates (100%).

In this study, 46.7% (28/60) of *Acinetobacter* isolates were positive for *bla*$_{\text{SIM-1}}$ gene. This finding agrees with the study of Alkasaby and Zaki[37], who detected *bla*$_{\text{SIM-1}}$ gene in 47.1% of *Acinetobacter* isolates in Mansoura University Hospital ICU. Inconsistent with our results, a published study by Nogbou et al.[34] declared that *bla*$_{\text{SIM-1}}$ was the least detected MBL gene (3%) among their *Acinetobacter* isolates.

In the current study 8.3% (5/60) of *Acinetobacter* isolates were positive for *bla*$_{\text{VIM}}$ gene. Similarly, Vijayakumer et al.[38], detected *bla*$_{\text{VIM}}$ in 5.7% of isolated *Acinetobacter* strains. However, several studies globally showed different rates for *bla*$_{\text{VIM}}$ gene. Alkasaby et al.[37] reported that no isolate was positive for the *bla*$_{\text{VIM}}$ gene. A published study in South Africa[34] stated that *bla*$_{\text{VIM}}$ was the most frequently detected gene (86%). Another study in Iran[28], declared that *bla*$_{\text{VIM}}$ was observed in 77% of the isolates.

In the present study *bla*$_{\text{IMP-1}}$ gene was not detected in any isolate. Concurring with our finding, a study by Baker et al.[39], reported that no *Acinetobacter* strain was positive for *bla*$_{\text{IMP-1}}$ gene. Additionally, Alshami et al.[28] in Iran stated that *bla*$_{\text{IMP}}$ was observed in none of the isolates. On the contrary, an Egyptian study reported that *bla*$_{\text{IMP-1}}$ gene was detected in 95.7% of *Acinetobacter* isolates[37].

In our study, many different combinations between the studied genes were detected in CRA isolates. The most prevalent combinations present were (*bla*$_{\text{OXA-51}}$ + *bla*$_{\text{OXA-23}}$) with percentages of 18.3%. These findings corroborate with another study, which reported that the (*bla*$_{\text{OXA-51}}$ + *bla*$_{\text{OXA-23}}$) combination was predominant between the carbapenem-resistant isolates[40].

CONCLUSION

CRA infections were considerable in our ICUs. The high prevalence of XDR *Acinetobacter* isolates observed in our study is very alarming and needs urgent intervention, strict adherence to infection control practices, and stop extensive and improper use of antibiotics particularly carbapenems in ICUs. Polymyxins have become the final existing therapeutic choice for XDR *Acinetobacter* infections in critically ill patients even though their neurotoxicity and nephrotoxicity side effects, that call for a critical need of new effective antibiotics against XDR *Acinetobacter* infections.

Institutional Review Board Statement: The study was approved by the Faculty of Medicine, Institutional Review Board, Assiut University, ethics committee (IRB approval number: 17101950, Date: 19/5/2020).

Conflicts of Interest: The authors declare no conflict of interest.

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

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