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Genetic Characteristics of Multidrug-Resistant *Acinetobacter baumannii* that Cause Nosocomial Infections Mainly in Intensive Care Units in Some Egyptian Hospitals

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Abstract

Nosocomial infections are one of the most common medical complications affecting patients admitted to the intensive care units (ICUs). This study aimed to investigate the multidrug-resistance (MDR), metallo-β-lactamases (MBLs) production in imipenem resistant (IR) species, detection of biofilm, and molecular characterization of MDR-associated genes in *A. baumannii* (*AB*) isolated from ICUs in Egypt. A total 77 (25.08%) *A. baumannii* isolates were recovered from 307 Gram negative bacteria (GNB) collected from different clinical specimens of infected cases hospitalized at SICU, MICU, CCU, NICU and PICU in 4 different Egyptian Hospitals. MDR were observed in 55/77 isolates out of them, 33 isolates were meropenem resistant (MR), while 35 isolates *were* imipenem resistant (IR). Out of 35 IR*AB* 19 isolates were MBL producers by CDT while 25 isolates were MBL producers by DDST. Strong biofilm production was observed in (20/33 isolates) of MR*AB*. All *A. baumannii* strains involved in this study produced the expected band of 353 bp, for *bla*OXA-51-like gene. Among the 19 CDT-positive *A. baumannii* isolates, 11 (57.9%) isolates were detected MBL encoding gene (*bla*NDM) and 18/23 (78.3%) of tested isolates were positive for *adeB* gene. This study revealed that MDR *A. baumannii* strains accommodate *bla*OXA-51**,** *blaNDM* and *adeB* genes were widely circulating in ICUs of Egyptian hospitals. In addition, the MDR biofilm and MBL production were observed among *A. baumannii* isolates that represents a serious public health problem.

Keywords: Multidrug-Resistant, *Acinetobacter baumannii*, Nosocomial Infections..

1. Introduction:

Acinetobacter baumannii causes a variety of nosocomial infections including, bloodstream infections, urinary tract infections, meningitis, and wound infections **(Shi, X., *et.al*, 2021).** *A. baumannii* is a Gram-negative opportunistic nosocomial pathogen causing clinical infections and outbreaks in healthcare settings especially in ICUs. This species account for almost 90% of all reported *Acinetobacter* infections, including respiratory tract infections, bacteraemia, meningitis, wound infections and urinary tract infections **(Abd El-Baky, R. M., *et.al*, 2020).** *A. baumannii* is able to easily acquire resistance to different groups of antimicrobials and to survive in hospital

environment, leading to its persistence and transmission in healthcare settings. Multidrug-resistant *A. baumannii* has become life threatening and is increasingly reported worldwide, including Europe, America, Asia and Africa **(Dijkshoorn L*, et. al,* 2007).**

In the hospitals in Egypt, carbapenems, such as meropenem and imipenem, are reserved for the treatment of the most severely ill patients due to their high potency, broad-spectrum activity, and good safety profile**.** Carbapenem resistance is one of the major threats challenged in antimicrobial treatment of infections caused by Gram negative organisms since these agents have been considered the last resort for effective therapy for MDR Gram-negative bacteria (**Abdulall, A. K.*, et.al*, 2018)**.

Resistance of *A. baumannii* to carbapenems is mainly mediated by the production of plasmid and chromosome-encoded carbapenem hydrolyzing β-lactamases, and reduced permeability as a result of porin loss or modification. Efflux pump overexpression and penicillin-binding proteins alteration have been found to play a role in developing resistance to carbapenems also. Resistance to carbapenems is most frequently mediated by the enzymatic hydrolysis of the drugs (**Benmahmod, A. B.*, et.al.* (2019)**).

In *A. baumannii* four types of MBL have been identified including, New Delhi metallo-β-lactamase (NDM), imipenemase (IMP), Seoul imipenemase (SIM) and Verona integron-encoded metallo-β-lactamase (VIM). Two variants (NDM-1 and NDM-2) of NDM have been documented in Egyptian *A. baumannii* clinical isolates. **(Abouelfetouh, A., *et. al* 2019)**.

The *bla*NDM-1gene has been detected on different large plasmids, which were readily transferable among bacteria, making NDM-1-producing bacteria a serious clinical and public health threat (Chen et al., 2011). Other clinical types include*bla*KPC, *bla*VEB, *bla*PER, *bla*BEL-1, *bla*BES-1, *bla*SFO-1, *bla*TLA, and *bla*BIC **(Dhillon and Clark 2012)).**

Efflux pumps are involved in resistance of *A. baumannii* isolates to antimicrobial agents. AdeABC efflux pump is one of the RND superfamily efflux pump and consists of *adeA* (membrane fusion), *adeB* (multidrug transporter) and *adeC* (outer membrane) genes **(Magnet, *et. al.* 2001).** Our study was aimed to detect the multidrug-resistance (MDR), metallo-β-lactamases (MBLs) production in imipenem resistant (IR) species, detection of biofilm, and molecular characterization of MDR-associated genes in *A. baumannii.* isolated from ICUs in Egypt.

2. Materials and methods

2.1. Specimen collection

A total of 77 clinical isolates of *A. baumannii* were recovered from different clinical specimens including blood, wound swab, urine, sputum, and Endotracheal aspiration (ETT) specimens from cases hospitalized at different ICUs as SICU, MICU, CCU, NICU and PICU at different departments of 4 different Egyptian Hospitals including, El- Hussein University Hospital, El-Azhar University Hospital, Al Zahra Hospital, and Sayed Galal Hospital in Cairo, Egypt during the period from August 2021 until July 2022.

2.2. Culture media used in the current study

In the current study, multiple culture media are used for collection, isolation, purification and preservation of *A. baumannii* species including, Nutrient agar, MacConkey agar, Tryptic soy agar, Triple sugar iron agar (TSI), Mueller-Hinton agar, Brain heart infusion broth Bile esculin media (all from Oxoid), Methyl red Voges-Proskauer broth (Difco Lab, USA). DNase agar medium, Tryptic soya broth, Simmons citrate agar, Amies transport medium without charcoal, Cetrimide agar and Christensen urea agar (all from LAB-M, UK.) were prepared according to the suppliers in structure at pH 6.8. Culture media are incubated at $37C^0$ for 24 hr.

2.3. Identification of clinical isolates of *A. baumannii*

2.3.1. Conventional biochemical tests

Isolates were identified according to **(Procop, *et, al.* 2017))**. Isolation and identification procedures were carried out using Gram staining and microscopic examination, colony characteristics, and conventional biochemical activities including catalase, Oxidase, DNase production, esculin hydrolysis, ornithine, and lysine decarboxylation, motility, citrate utilization, and triple sugar iron agar (TSI).

2.3.2. API 20E System

The identified *Acinetobacter* species isolates were confirmed by API 20E system (BioMerieux, France). These tests were used according to manufacturer's protocol for Enterobacteriaceae and non-enteric bacteria. Wells of biochemical test were inoculated with overnight 0.5 McFarland bacterial suspension and incubated at 37oC for 24 hrs. The results were read after addition of reagents, as 7-digit number that identifies by API 20E analytical index.

2.3.3. Molecular identification method

2.3.3.1. PCR assay for *bla*OXA-51-like gene.

PCR amplification of *bla*OXA-51-like gene was conducted using primer in **Table (1)**. In 25 µl reaction volumes and the components of the reaction mixture for each case of genomic DNA amplification were as in **Table (2)**.

Table (1): List of sequencing of primers used in PCR for molecular identification and detection of antimicrobials resistance-associated genes of *A. baumannii.*

Target(s)	Primer	Sequence 5' to 3'	Expected amplicon size	References
***bla*OXA-51-like**	***OXA-51-like-F***	**5' -TAATGCTTTGATCGGCCTTG-3'**	**353bp**	**(Daef *et al.*, 2012)[17]**
***bla*OXA-51-like**	***OXA-51-like-R***	**5' -TGGATTGCACTTCATCTTGG-3'**		
***Ade*ABC efflux pump**	***adeB F***	**5-TTAACGATAGCGTTGTAACC-3**	**541 bp**	**(Lina *et al.*, 2008)[25]**
	adeB R	**5-TGAGCAGACAATGGAATAGT-3**		
***bla*VIM**	***VIM -F***	**5'- GATGGTGTTTGGTCGCATA-3'**	**390 bp**	**(Dallenne *et al.*, 2010)[23]**
	VIM -R	**5'-CGAATGCGCAGCACCAG-3'**		
***bla*IMP**	***IMP-F***	**5'-TTGACACTCCATTTACDG-3'**	**139 bp**	**(Dallenne *et al.*, 2010)[23]**
	IMP -R	**5'-GATYGAGAATTAAGCCACYCT-3'**		
***bla*NDM**	***NDM- F***	**5'-CCCGGCCACACCAGTGACA-3'**	**129 bp**	**(Voets *et al.*, 2011)[24]**
	NDM -R	**5'- GTAGTGCTCAGTGTCGGCAT-3'**		
***bla*SIM**	***SIM -F***	**5'- TTGCGGAAGAAGCCCAGCCAG-3'**	**613 bp**	**(Voets *et al.*, 2011)[24]**
	SIM -R	**5'-GCGTCTCCGATTTCACTGTGG-3'**		
***bla*GIM**	***GIM-F***	**5'-CGTTGCCAGCTTTAGCTCAGG-3'**	**279 bp**	**(Voets *et al.*, 2011)[24]**
	GIM- R	**5'-GCAACTTGATACCAGCAGTGCG-3'**		
***bla*SPM**	***SPM -F***	**5'- GGGTGGCTAAGACTATGAAGCC-3'**	**447 bp**	**(Voets *et al.*, 2011)[24]**
	SPM – R	**5'-GCCGCCGAGCTGAATCGG-3'**		

Table (2): The components of the reaction mixture for each case of genomic DNA amplification and detection of *bla*OXA-51-like gene.

Component	Volume
10x buffer	**2.5 µl**
50 mM $MgCl_2$	**1.0 µl**
25 mM dNTPs	**0.5 µl**
10 uM primer	**10.0 µl**
5 U/µl Taq polymerase	**0.2 µl**
10 ng/µl template DNA	**3.0 µl**
Nuclease free water	**7.8 µl**
Total volume	**25 µl**

The conditions for the PCR were 95ºC for 4 min and then 34 cycles at 95ºC for 45 sec, 34 cycles at 52ºC for 45 sec, and 34 cycles at 72ºC for 30 sec. This was followed by a final extension at 72ºC for 10 min.

2.3.3.2. Separation of the PCR product by agarose gel electrophoresis.

Gel electrophoresis was used for the detection of DNA and PCR products which visualized with the aid of ethidium bromide and UV transilluminator **(Sambrook, et. al, 1989))**.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of bacterial isolates was performed for *A. baumannii* isolates by the disc diffusion method according to Kirby-Bauer technique (1966) according to Clinical and Laboratory Standards Institute **(CLSI, 2017)**. The tested agents included Ceftazidime (CAZ), Aztreonam (ATM), Amikacin (AK), Ampicillin-Sulbactam (SAM), Chloramphenicol (C), Piperacillin-Tazobactam (TZP), Piperacillin (PRL), Ceftriaxone (CRO) Ciprofloxacin (CIP), Gentamicin (CN), Imipenem (IPM), Levofloxacin (LEV), Sulfamethoxazole-Trimethoprim (SXT), Nitrofurantoin (F).

2.5. Phenotypic Methods for Detection of Metallo-β-lactamases (MBL)

2.5.1. Imipenem EDTA Double Disc Synergy Test (DDST)

Double disc synergy test was done as described by **(Lee, *et. al.* 2003).** Three to five well

isolated colonies of same morphology were selected. A 0.5 McFarland solution was made and with the help of a swab the inoculum was applied to a MHA plate with the help of a rotor. A 10 µg imipenem disc was applied to the plate. Another sterile filter paper disc was then applied 10 mm away to the imipenem disc (edge to edge). 10 µl of 0.5M EDTA solution was then added to the filter paper disc (750µg). The plates were then incubated and the results were interpreted after 16-18 hours. The tests were interpreted as positive if a large synergistic zone was formed in between the discs.

2.5.2. Imipenem-EDTA Disc Method (Combined Disc Method)

Combined disc test (CDT) was done as described by **(Yong, *et. al*, 2002).** This test was done to confirm the production of MBLs phenotypically. Three to five well isolated colonies of same morphology were selected. A 0.5 McFarland solution was made and with the help of a swab the inoculum was applied to a MHA plate with the help of a rotor. Two imipenem discs (10 µg) were applied apart to the plate. To one of the discs 10 µl of 0.5M EDTA solution (750 µg) was then added. The plates were then incubated and the results were interpreted after 16-18 hours. The test was interpreted as positive for MBL if the difference in zones of inhibition of ≥7mm is produced.

2.6. Detection of biofilm formation.

Microtiter Plate (MTP) technique was used for asses' biofilm formation for IRAB isolates as formerly described by **(Amoli, 2017)**).

2.7. Multiplex polymerase chain reactions for detection of MBLs encoding genes in IR*AB*

Taking in account either the necessity to have an easy interpretation (avoiding similar-in-size amplicons for one given PCR tube), but also the clinical relevance of the screening (combining together the most widely distributed genes in clinical isolates), 2 multiplex reactions were defined, with No.1 including detection of (*bla*GIM, *bla*NDM, *bla*SPM, and *bla*SIM), No. 2 including detection of (*bla*IMP and *bla*VIM) genes in *A. baumannii* isolates as described by**. (23, Voets, e. al, 2011).** Amplification was done by conventional PCR for detection the presence of *bla*GIM, *bla*NDM, *bla*SPM, *bla*SIM, *bla*IMP and *bla*VIM genes using specific primers as shown in **Table (2).** PCR was carried out in 25 µL reactions using 12.5 µL of DreamTaq Green PCR Master Mix (2X), 1 µL of each primer, 3µL (20 ng) of template DNA and PCR grade water to complete 25 µL. The PCR temperature conditions include: 95 ^{0}C for 1 min, followed by 35 cycles of 95 ^{0}C for 30 sec, 60 ^{0}C for 30 sec for multiplex 1 and 55 ^{0}C for 30 sec for multiplex 2 followed by 72 ^{0}C for 1 min and final extension at 72 ^{0}C for 7 min.

2.8. Statistical analysis

All data analyses were performed where categorical variables were compared using the Fisher's exact test. *P* value ≤ 0.05 was considered statistically significant. All the statistical analyses were performed using GraphPad Prism version 8.0.2.

2. Result and Discussion

A total 77 (25.08%) *A. baumannii* isolates were recovered from 307 Gram negative bacteria (GNB) collected from different clinical specimens of infected cases hospitalized at SICU, MICU, CCU, NICU and PICU in 4 different Egyptian Hospitals.

2.1. Identification of bacterial isolates

2.1.1. Conventional biochemical tests

Acinetobacter baumannii isolates were non-motile bacteria, and were able to grow at 44 ^{o}C and displayed positive ornithine decarboxylation reaction as showed in **Table (3)**.

2.1.2. API 20E System (BioMerieux, France)

Wells of biochemical test were inoculated with overnight 0.5 McFarland bacterial suspension and incubated at 37 ^{o}C for 24 hrs. The results were read after addition of reagents, as 7-digit number that identifies by API 20 analytical index**.**

2.2. Detection of antimicrobial susceptibility pattern among *A. baumannii* isolates.

Antimicrobial susceptibility profile of 77 isolates of *A. baumannii* showed that 90% of the isolates were resistant to ceftriaxone and showed a high level of resistance against piperacillin, piperacillin-tazobactam, ceftazidime, and ampicillin-sulbactam (88% for each), ciprofloxacin (80%), levofloxacin and amikacin (61%). Intermediate bacterial resistance was found against Imipenem (24%) and amikacin (14%). While, sulfamethoxazole-trimethoprim (44%) showed the highest level of sensitivity followed by imipenem and levofloxacin (37% for each) and aminoglycosides (25%) for amikacin and (24%) for gentamycin as shown in **Figure (1)**.

2.3. **Incidance of recovered of MDR isolates of *A. baumannii***

Among 77 *A. baumannii* isolates, 55 were MDR out of them resistance to more than three different classes of antibiotic. B-lactams were the most common resistant classes of antibiotics for all MDR isolates followed by Aminoglycosides, Folate pathway inhibitors and Quinolones. As shown in **Figure (2).**

3.4. Incidence of Carbapenems (Meropenem & Imipenem) resistant isolates among *A. baumannii*.

Detection of carbapenems (meropenem & imipenem) resistant (MR-IR) isolates was

Table (3): The biochemical reactions of the isolated *A. baumannii* bacteria.

Item	Test		*A. baumannii*
1	Motility		Negative
2	Fermentation / oxidation on Hugh and Leifson's medium		Oxidative
3	Catalase		Positive
4	Oxidase		Negative
5	DNase		Negative
6	Ability to grow on 44 ºC		Positive
7	Lysine decarboxylation		Negative
8	Ornithine decarboxylation		Positive
9	Citrate utilization		Positive
10	Indole production		Negative
11	Urease production		Negative
12	Nitrate reduction		Negative
13	Methyl red		Negative
14	Voges-Proskauer		Negative
15	Triple sugar iron agar	Slant	Alkaline
		Butt	Alkaline
		H2S production	Negative
		Gas production	Negative
16	Gelatin Hydrolysis		Negative
17	Fermentation of Maltose		Negative
18	Fermentation of Mannitol		Negative
19	Esculin hydrolysis		Negative

performed. Among the 77 isolates of *A. baumannii*, 33 isolates (42.86%) were resistant to meropenem. While, 35 isolates (45.45%) were resistant to imipenem. As showed in **Figure (3).**

3.6. Biofilm formation

The biofilm pattern of MR*AB* isolates were screened for biofilm production spectrophotometrically using microtiter plate method. 25/33 MR*AB* isolates (71.43%) showed biofilm-forming ability. Biofilm production levels varied among different isolates strong biofilm producers 20/25 (80.0 %) were the most common biofilm pattern noticed among MR*AB* while the remaining isolates showed 12% moderate and 8% weak biofilm forming ability**.**

3.7. Detection of metallo beta Lactamases (MBLs) producing isolates in *A. baumannii*

3.7.1. Imipenem EDTA Double Disc Synergy Test (DDST)

In DDST, out of 35 of ***A. baumannii*** 25 isolates (71.43%) were DDST positive (MBL producers). **Imipenem-EDTA Disc Method (Combined Disc Test)**

Metallo-β-lactamase production was phenotypically detected by Imipenem-EDTA combined disc test (CDT) technique**.** Out of 35 of ***A. baumannii*** 19 isolates (54.29%) were MBL producers.

3.8. Molecular Study:

In this study, confirming identification of *A. baumannii* using *bla*OXA-51-like gene, and molecular detection some MBL encoding genes (*bla*IMP, *bla*VIM *bla*GIM, *bla*NDM, *bla*SPM, and *bla*SIM) in *A. baumannii* isolates and distribution of *adeB* gene in meropenem-resistant isolates of *A. baumannii*.

3.8.1. Confirming identification of *A. baumannii* using *bla*OXA-51-like gene-directed PCR

All strains involved in this study produced the expected band of 353 bp, a band for *bla*OXA-51-like gene and therefore identified as *A. baumannii* **(Figure 4)**.

3.8.1. Molecular detection of MBL encoding genes in *A. baumannii*

The multiplex polymerase chain reactions (PCR) were used to detect the presence of some MBL encoding genes (*bla*IMP, *bla*VIM *bla*GIM, *bla*NDM, *bla*SPM, and *bla*SIM) in *A. baumannii* isolates. Among the 19 (32.2%) CDT-positive *A. baumannii* isolates, 11 (57.9%) isolates were contened MBL encoding gene (*bla*NDM). PCR

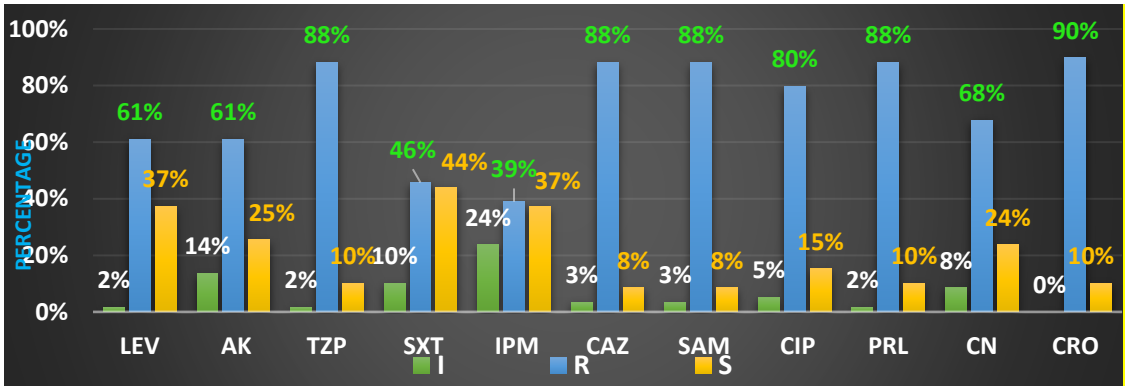

Figure (1): Antimicrobial susceptibility patterns of *A. baumannii* isolates.

Ceftazidime (CAZ), Amikacin (AK), Piperacillin-Tazobactam (TZP), Piperacillin (PRL), Ceftriaxone (CRO) Ciprofloxacin (CIP), Gentamicin (CN), Imipenem (IPM), Levofloxacin (LEV), Sulfamethoxazole-Trimethoprim (SXT), Ampicillin-Sulbactam (SAM).

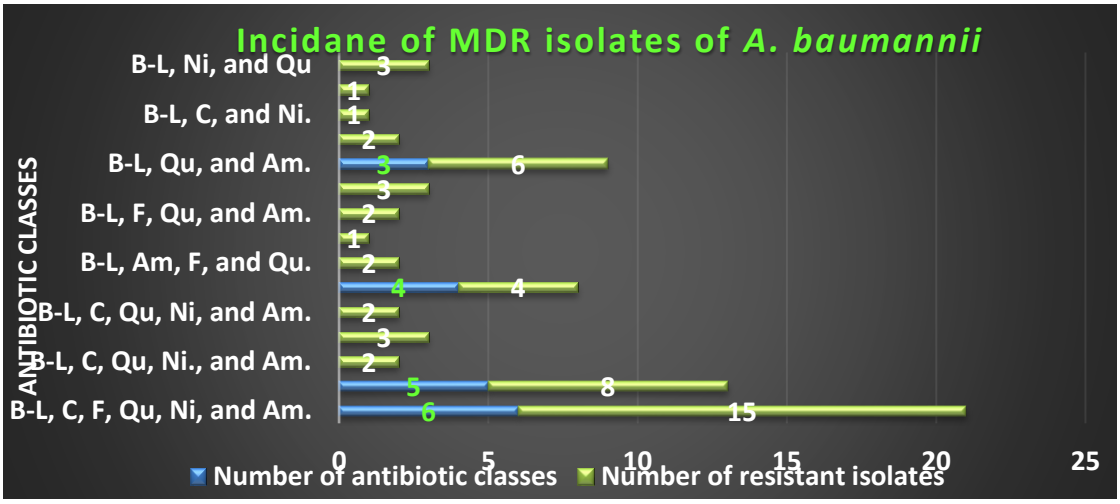

Figure (2): Frequency of recovered MDR isolates of *A. baumannii*

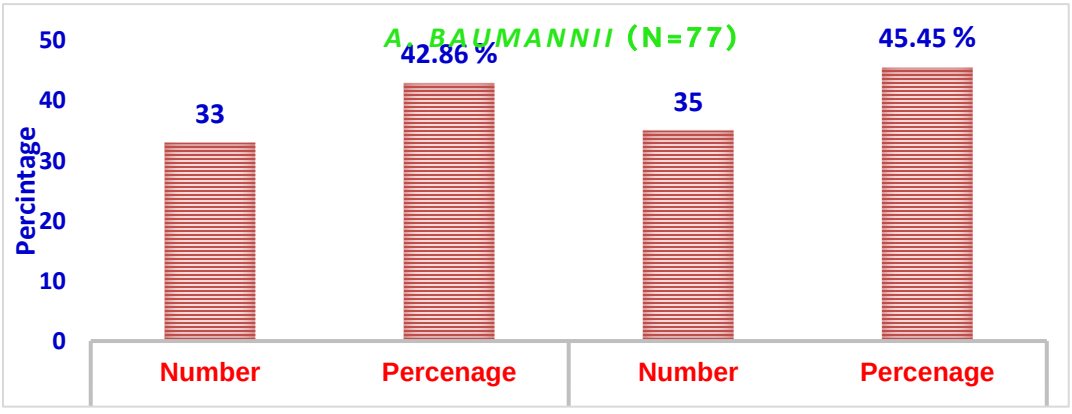

Figure 3: Incidence of Carbapenems (MR & IR) isolates among *A. baumannii*

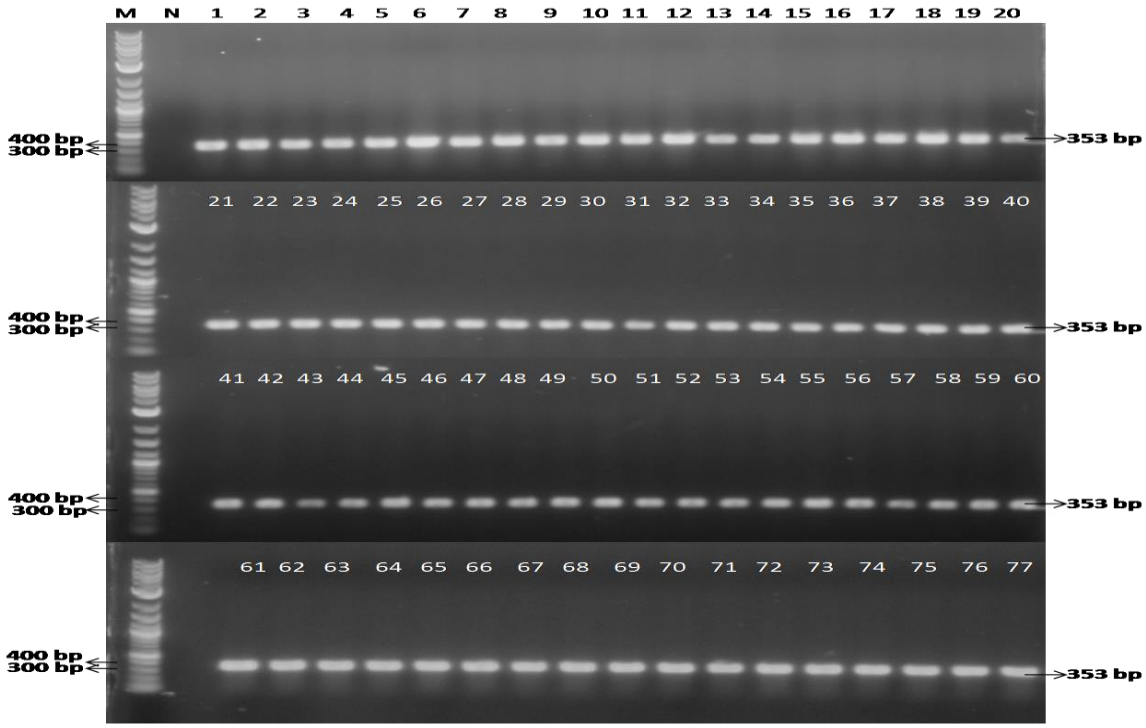

Figure (4): PCR detection of *bla*OXA-51-like gene. M; DNA marker, lane N; negative control and lanes 1-77; positive results of *A. baumannii* isolates investigated.

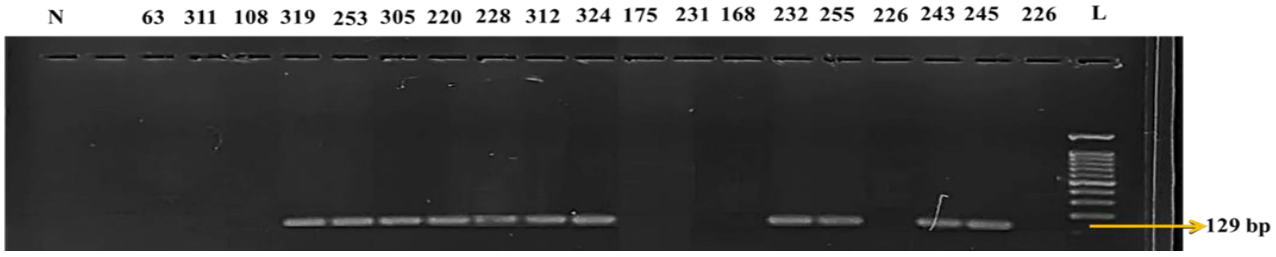

Figure (5): Agarose gel electrophoresis of *bla*NDM gene amplified from 19 *A. baumannii* isolates using 100 bp DNA marker. N: negative control.

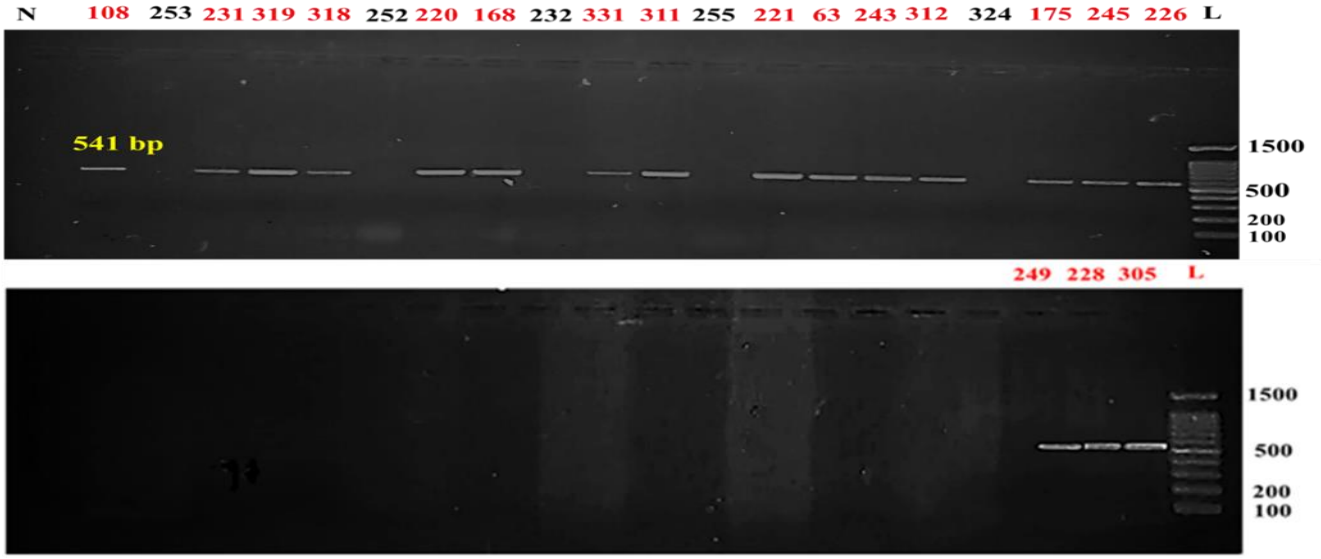

Figure (6): Agarose gel electrophoresis of *adeB* gene amplified from 23 meropenem-resistant *A. baumannii* isolates using 100 bp DNA marker. N: negative control.

detected amplification of a 129 bp fragment corresponding to the *bla*NDM gene and no isolates showing the presence of other tested MBL genes (*bla*IMP, *bla*VIM *bla*GIM, *bla*SPM, and *bla*SIM) as demonstrated in **Figure (5)**.

3.8.1. Molecular detection of *adeB* gene in meropenem-resistant isolates of *A. baumannii.*

In this study a total of 23 meropenem-resistant *A. baumannii* isolates were investigated for the presence of AdeABC efflux pump genes using conventional PCR and specific primer for *adeB* gene, 18/23 (78.3%) of tested isolates were positive for *adeB* gene as they showed the expected amplicon of 541 bp. The results of PCR gel electrophoresis are shown in **Figure (6)**.

3.8.1.1. Distribution of *adeB* gene in meropenem-resistant *A. baumannii* among ICUs

The distribution of *adeB* gene in meropenem-resistant *A. baumannii* isolates among several ICUs showed that the highest percentages of *adeB gene* were isolated from SICU followed by PICU, CCU, MICU and NICU respectively as showed in **Figure (7).** *Acinetobacter baumannii* is a non-motile, encapsulated, non-lactose fermenting Gram-negative coccobacillus. It is widely spread in nature, hospital environments, the skin surface of humans, and organs such as the intestinal, respiratory, and urinary tract **(Abd El-Baky, *et. al,* 2020)**. *Acinetobacter* accounts for approximately 2% of hospital acquired infections in the USA but these rates are doubled in Asia and the Middle East with up to 20% of ICUs worldwide. *A. baumannii* has proven to be an increasingly important and demanding species in HAIs in Egyptian hospitals. *A. baumannii* was isolated from at least 10% of nosocomial infections in ICUs in many Cairo hospitals **(Farid, *et. al,* 2016)**.

In the present study, a total 77 (25.08%) *A. baumannii* isolates were recovered from 307 Gram- negative bacteria (GNB) collected from different clinical specimens of infected cases hospitalized at SICU, MICU, CCU, NICU and PICU in 4 different Egyptian Hospitals. This result was in accordance with other previous studies from Egypt, **(El-Masry, and El-Masry, 2018),** they found that *A. baumannii* represent (16.1% and 17.4%) of total collected, while other Egyptian studies conducted by **(Mabrouk, *et. al,* 2020)** reported higher prevalence rate (37.7%). On the other hand, lower prevalence rate (4.6% and 3.65 %) was recorded by **(Hassan, *et. al*. 2021)** respectively. The difference between the current study and other studies could be related to environmental conditions, source of specimens, health practices, patient conditions, personal hygiene, number of patients involved in each study, and/or laboratory procedures.

In our study, high level of resistance (>85%) were detected against most of β-lactams especially Cephalosporins and β-lactam combination which are the most frequent prescribed antibiotics in our hospitals, our results were in consistent with concluded by other former Egyptian studies, **(Khodier, *et. al.* 2020),** they documented high prevalence of Cephalosporins resistant among clinical isolates of *A. baumannii*.

In the study, among 77 *A. baumannii* isolates 55 (71.43%) were MDR. Similar high multidrug resistance rate was detected by **(Sievert, *et. al,* 2013)** in the US National Healthcare Safety Network they reported that 70% of *Acinetobacter* species, was MDR, while in Iran, **(Mirzaei, *et. al,* 2020))** reported that 74.75% of *A. baumannii* were MDR, in addition an Indian study, **(Solanki, *et. al,* 2020)** reported that MDR was detected in 68.42% of *Acinetobacter* strains, in study, a multicentre study **(See, *et. al,* 2013)** conducted in 46 ICUs in 11 Egyptian hospitals, 100% of *Acinetobacter* strains were MDR also in another Egyptian study, **(Hassan, *et. al*. 2021)** observed that *Acinetobacter*-MDR isolates were100%.

In 2017, the WHO published a list of 'priority pathogens' for which new antibiotics are urgently needed, with the highest priority being given to carbapenem-resistant *A. baumannii* (CRAB), carbapenem-resistant *P. aeruginosa* (CRPA), and carbapenem-resistant and third generation-cephalosporin-resistant *Enterobacteriaceae*. The rapid emergence of carbapenem resistant *A. baumannii* is considered a substantial health problem globally due to limited therapeutic options (**Benmahmod, A. *et. al.* 2019)**.

In the current study, MDR were observed in 55/77 isolates out of them, 33 isolates were meropenem resistant (MR), while 35 isolates *were* imipenem resistant (IR). Our results were matched with previous studies conducted in Egypt, **(Abd El-Baky, *et.al,* 2020)** they detected emergence of resistance against imipenem among *Acinetobacter* species (25% and 20%), also another Indian study, **(Solanki, *et. al,* 2020)** reported that 23% of *Acinetobacter* species were Imipenem resistant. On the other hand, much, higher rate was reported by other earlier researchers in Egypt and in other countries**., (Alkasaby, & El Sayed, 2017)** in Egypt, they observed that 95.7% of *A. baumannii* isolates were Imipenem resistant, **(Hashemi, *et. al,* 2020)** in Iran, found that 88% of *A. baumannii* isolates were resistant to Imipenem, **(Agarwal, *et. al,* 2017)** in India and **(Shi, X., *et.al,* 2021)** in China concluded that meropenem resistance *A. baumannii* isolates was 90.5% and 91.5% respectively. The emergence of *A. baumannii*

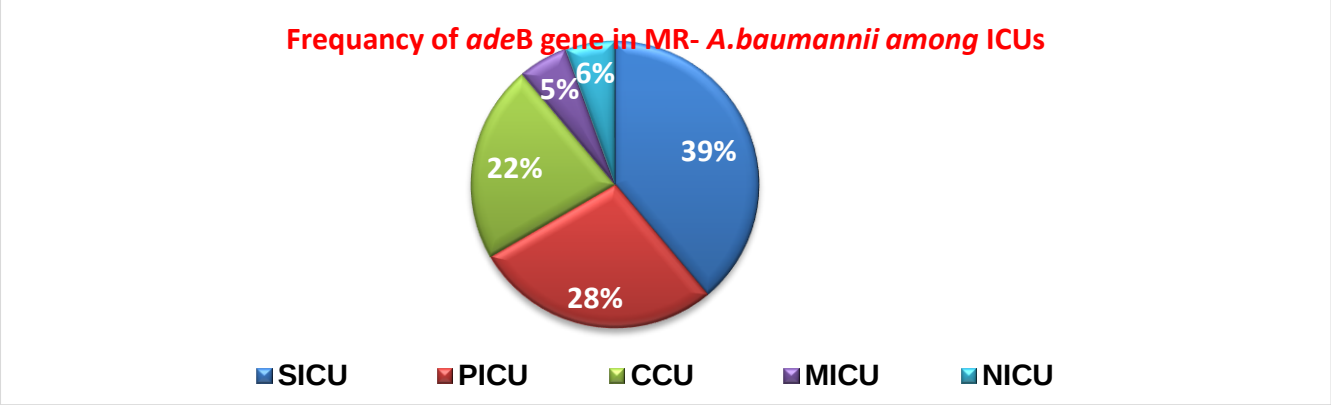

Figure (7): Frequency of *adeB* gene in meropenem-resistant *A. baumannii* among various ICUs

strains with increased carbapenem resistance may be due to the excess misuse of carbapenems in addition to inadequate adherence to infection control guideline.

The most dominant mechanism of carbapenem resistance in *A. baumannii* is the enzymatic degradation by carbapenem hydrolyzing β-lactamases **(Al-Agamy, et. al, 2014)** The most common carbapenemases in *A. baumannii* are the carbapenem hydrolyzing class D β-lactamases and MBL, while Class A carbapenemases were found at a lesser extent (**Khodier, *et. al.* 2020)**. MBLs have the ability to hydrolyze almost all clinically available β-lactam antibiotics. MBLs are compromising the therapeutic efficacies of β-lactams, particularly Carbapenems, which are last-resort antibiotics indicated for various multidrug-resistant bacterial infections **(Solanki, *et. al,* 2020)**. Molecular and non-molecular methods have been used for screening bacterial isolates for MBL production. (**Mohamed, *et. al*, 2011)**.

In the present study in DDST, out of 35 of *A. baumannii* 25 isolates (71.43%) were DDST positive (MBL producers) and out of 35 of *A. baumannii* 19 isolates (54.29%) were MBL producers. This result was in accordance with that of **(Abo-Alella, *et. al*, 2021)** who reported that 80% of carbapenem resistant *A. baumannii* isolates collected from Egyptian patients were MBL producers by the CDT. Similar results were obtained with studies conducted by **(Shivaprasad, *et. al,* 2014)**, they observed that (81.18%) of isolates were recognised as MBL producers by CDT and DDST. Higher rate of expression of MBLs resistance in *A. baumannii* isolates demonstrate that it has become a significant contributing factor for imipenem resistance among *A. baumannii* species.

All strains in this study produce the expected band of 353 bp, a band for *bla*OXA-51-like gene and therefore identified as *A. baumannii*. All isolates identified as *A. baumannii* in this study, were furthermore confirmed by producing the specific band of blaOXA-51-like gene in PCR investigations using oligonucleotides targeting this *A. baumannii* unique gene. These results agree with **(Feizabadi, *et. al,* 2008)** who reported that all *A. baumannii* isolates were positive for blaOXA-51-like gene in PCR amplification; however other *Acinetobacter* species were not.

Molecular-based techniques continue to be the golden and best method to confirm the presence of MBL genes. Several MBL types, including, IMP, VIM, SPM-1, GIM-1, SIM-1, NDM-1, DIM-1, TMB-1 and KHM-1 are identified in GNB worldwide **(Farrag, *et. al*, 2015)**. Regarding the genotypic analysis, multiplex PCR was used to ensure the presence of some MBL encoding genes (bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{SIM} and bla_{GIM}) responsible for imipenem resistance in *A. baumannii* isolates in the present study. PCR revealed amplification of a 129 bp fragment related to the bla_{NDM} gene in 11/19 (57.9%) CDT-positive *A. baumannii* isolates, while none of the isolates showed amplification of other genes. This outcome was matched with several previous studies done in Egypt that recorded high incidence and predominance of bla_{NDM} gene in *A. baumannii* and other GNB isolates recovered from Egyptian hospitalized patients, **(El-Domany, *et. al*, 2021)** they reported that the bla_{NDM} gene was found in high prevalence (39.3%, 66.6%, 68.2%, 70.0% and 88.4%) respectively, compared to other genes revealing the emergence of the dissemination and rapid spread of this gene over different regions in Egypt.

In an Egyptian study conducted by **(Al-Agamy, *et. al,* 2014)**, observed that, among 40 imipenem resistant *A. baumannii* isolates MBLs was not identified to play a role in carbapenem resistance in *A. baumannii* isolates. On contrary side in the current study eleven *A. baumannii* isolates were harboured bla_{NDM} gene.

In our study a total of 23 meropenem-resistant *A. baumannii* isolates were investigated for the presence of AdeABC efflux pump genes using conventional PCR and specific primer for *adeB* gene, 18/23 (78.3%) of tested isolates were positive for *adeB* gene. The AdeABC efflux pump is one of the important antibiotic resistance mechanisms which are applied by Gram-negative bacteria such as *A. baumannii*. In recent studies, it has been approved that 80% of MDR *A. baumannii* isolates showed close correlation between resistance and AdeABC pump genes **(Lina *et. al.* 2008).** It was proved that AdeABC pump is responsible for increasing resistance to common antibiotics which act as substrates for efflux pump such as tetracycline **(Wieczorek *et. al,* 2008).**

Conclusion

The results of this study provide evidence for appropriate surveillance and outbreak investigations. The presence MDR *A. baumannii* isolates are becoming a serious issue in ICUs, with many strains developing resistance to most available antibiotics. This study concluded the MDR, biofilm and MBL production were observed among *A. baumannii* isolates that represents big public health problem. In addition, MDR *A. baumannii* strains harbouring *blaNDM* and *adeB* genes were widely circulating in certain ICUs of Egyptian hospitals. We propose that infection control measures and strict antimicrobial stewardship policies should be applied to reduce the selective pressure that inevitably favours the emergence and epidemic of MDR strains and to increase the therapeutic benefits of these antibiotics.

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