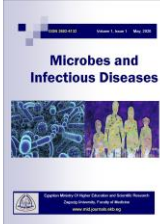
Microbes and Infectious Diseases 2024; 5(4): 1461-1470



Microbes and Infectious Diseases

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

Original article

Dissemination of NDM-mediated carbapenem resistance in biofilm-forming *Acinetobacter baumannii* in some Egyptian hospitals

Mohammed H. Gaballah*[*1], Hamido M. Hefny, Moselhy S. Mansy[1], Reem M. Hassan[2]

1. Microbiology & Immunology Department, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo, Egypt

2. Clinical Pathology Department, Faculty of Medicine, Cairo University, Egypt

ARTICLE INFO

Article history:

Received 23 July 2024

Received in revised form 4 September 2024

Accepted 11 September 2024

Keywords:

Acinetobacter baumannii

Class 1integron

MBL

Multiplex PCR

Biofilm formation.

ABSTRACT

Background: Carbapenem-resistant A. baumannii pose a serious threat, limiting treatment options for severe infections induced by this emerging pathogen. This study aimed to evaluate the antimicrobial resistance profile and detect resistance mechanisms in imipenem-resistant A. baumannii from various Egyptian hospitals. Materials and **Methods:** A total of 59 A. baumannii isolated from various clinical specimens (blood, sputum, wound swap, urine and endotracheal tubes) collected from the microbiology lab of different Egyptian hospitals. Isolates were identified using conventional bacteriological methods. Antimicrobial susceptibility was tested using Kirby-Bauer disc diffusion method. Biofilm formation was determined spectrophotometrically using microtiter plate method. Metallo-β-lactamase (MBL) resistance was determined by combined disk test (CDT). Multiplex PCR evaluated MBL-encoding genes in imipenem-resistant isolates. Additionally, PCR was used to detect class 1 integrons in MBL-producing isolates. **Results:** Strong biofilm production was observed in 74% (17/23) of isolates. Class 1 integron was detected in 78.9 % (15/19) of MBL producing isolates. Out of 23 isolates 19 (32.2%) were MBL producers by CDT. Among the 19 (32.2%) CDT-positive isolates, 11 (57.9%) isolates harboured MBL encoding gene (*bla*NDM). **Conclusion:** Our findings highlight the emergence and dissemination of *bla*NDM in our hospitals, coupled with their biofilms and MBL production ability, represents a major public health challenge. The presence of class 1 integrons in all isolates carrying MBL-encoding gene, suggesting the critical role of Class 1 integrons in the dissemination of resistance genes.

Introduction

Acinetobacter baumannii is a major cause of various hospital-acquired infections [1]. Carbapenem resistance in *A. baumannii* is mainly attributed to the production of carbapenem-hydrolyzing β-lactamases encoded on plasmids and chromosomes as well as reduced permeability due to alterations or absence of porins [2].

Four distinct types of MBL have been detected in *A. baumannii*: Verona integron-encoded metallo-β-lactamase (VIM), Imipenemase (IMP), Seoul Imipenemase (SIM), and New Delhi metallo-β-lactamase (NDM) [3]. *Acinetobacter* species have shown the presence of multiple RND efflux systems. Among them, AdeABC, originally found in a multidrug-resistant (MDR) isolate of *A. baumannii*,

DOI: 10.21608/MID.2024.306729.2097

* *Corresponding author:* Mohamed Hamza

E-mail address: *mohamed.hamza@azhar.edu.eg*

© 2020 The author (s). Published by Zagazig University. This is an open access article under the CC BY 4.0 license **https://creativecommons.org/licenses/by/4.0/.**

is known for its role in increasing resistance to several antimicrobial agents [4].

Acinetobacter baumannii has the ability to develop biofilm on a range of surfaces, both abiotic and on host epithelial cells [5]. Antimicrobial resistance associated with biofilm formation is a major concern in clinical practice, especially for biofilm-related infections [6]. Class 1 integrons, along with their gene cassettes, play a significant role in causing multidrug resistance in *A. baumannii* [7].

This study aimed to assess the antimicrobial resistance profiles and detection of some resistance determinants in imipenem-resistant *A. baumannii* isolates collected from various hospitals across Egypt.

Materials and methods

Bacterial isolates and antimicrobial susceptibility testing

In this study, 59 *A. baumannii* isolates were isolated from various clinical specimens, including blood, sputum, wound swap, urine and endotracheal tubes specimens collected from different Egyptian hospitals including Kasr Al-Ainy Hospital, Sayed Galal Hospital, and EL-Demerdash Hospital, during the period from January 2017 to October 2019, and identified using conventional bacteriological methods involving, Gram staining and microscopic examination, colony characteristics, and biochemical activities including catalase, Oxidase, DNase production, esculin hydrolysis, ornithine, and lysine decarboxylation, motility, citrate utilization, and triple sugar iron agar (TSI). Antibiotic susceptibility was tested using the standard Kirby-Bauer disc diffusion technique [8]. Bacterial isolates were cultured, standardized to a 0.5 McFarland turbidity, and inoculated onto Mueller-Hinton agar plates. Antibiotic-impregnated disks were applied, and plates were incubated. Zones of inhibition were measured and interpreted according to CLSI 2017 guidelines to determine antimicrobial susceptibility patterns [9]. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as internal quality control strains, according to the CLSI protocol.

Quantitative detection of biofilm formation.

Microtiter Plate technique was used for quantitative assessment of biofilm formation for IRAB isolates [10]. The bacteria were cultured in LB broth medium for 18-24 hours to investigate the biofilm and after this period, a bacteria suspension with opacity equivalent to a tube of 0.5 McFarland standard was prepared. To establish a baseline for biofilm formation, sterile distilled water was used as a negative control. 180 µl of Luria-Bertani broth medium was transferred to 96-well microplates and then, 20 µl of bacteria suspension was added to the wells and incubated at 37°C for 24 hours. After this period, inoculations were removed and washed with distilled water 3 times. the plates were dried for 30 min at 37°C. Then, 200 µl crystal violet was added for 5 min. it was washed with distilled water for 3 times and 200 µl of 30 % glacial acetic acid was added to each well and light absorption at 490 nm was read using a microplate reader. Three replicates were considered for each sample, and they were categorized as shown in Table (1).

Phenotypic detection of MBLs

Imipenem-EDTA combined disc test (CDT) was used to detect MBL production in IRAB isolates [11]. Imipenem resistant *A. baumannii* isolates were inoculated onto Mueller-Hinton agar as lawn culture. On the plate, two 10-µg imipenem disks (Hi-Media Laboratories, BD Diagnostics Pvt. Ltd.) were inserted and 10 µL of EDTA solution were added to one of them to achieve the required concentration (750 µg). The two Imipenem discs were positioned on the plate 20 mm center to center. Plates were subsequently incubated for 16–18 hours at 37°C in the air, MBL producer was defined as a zone of inhibition that was increased by Imipenem + EDTA disc by 7 mm as compared to Imipenem disc alone.

Multiplex polymerase chain reactions (1 & 2) for detection of MBLs encoding genes in IRAB

Two 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 [12,13]. Amplification was done by conventional PCR for detection of the presence of MBL encoding 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.

Detection of class 1 integron using PCR in IRAB

Template DNA from 23 imipenem-resistant Acinetobacter baumannii isolates was extracted using boiling method [14]. The existence of class 1 integron was determined by using primers specific for class I integrase (intI1) gene. The PCR was conducted in a 25 µl reaction mixture comprising 1 µl of template DNA, 0.8 µl of each forward (5'-CCCGAGGCATAGACTGTA-3') and reverse (5'-CAGTGGACATAAGCCTGTTC-3') gene primers, 12.5 µl of DreamTaq Green PCR master mix (2X) and 9.9 µl of PCR grade water. The PCR cycling parameters included: 95 ^{0}C for 1 min, followed by 35 cycles of denaturation at 95 ^{0}C for 30 sec, 30 sec of annealing at 59 ^{0}C for and 1 min of extension at 72 ^{0}C with final extension for 7 min at 72 ^{0}C.

Molecular detection of *adeB* gene

Molecular detection of AdeABC efflux pump in 23 IRAB was performed using primer for *adeB* gene specific for *A. baumannii* [15]. PCR reaction was carried out in 25 µL reactions using 12.5 µL of DreamTaq Green PCR Master Mix (2X), 1 µL for each forward (5'-TTAACGATAGCGTTGTAACC-3') and reverse (5'-TGAGCAGACAATGGAATAGT-3') gene primers, 2µL (25 ng) of template DNA and PCR grade water to complete 25 µL. The PCR cycling parameters included: 95 ^{0}C for 5 min, followed by 30 cycles of denaturation at 95 ^{0}C for 30 sec, 1 min of annealing at 55 ^{0}C and 6 min of extension at 72 ^{0}C with final extension for 10 min at 72 ^{0}C.

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.

Results

In the present study, fifty-nine (16.8%) isolates of *A. baumannii* were identified using conventional biochemical tests. Antimicrobial susceptibility profile of fifty-nine 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 amikacin (61%) while 38.95 (23/59) *A baumannii* isolates were Imipenem resistant. Intermediate bacterial resistance was found against imipenem (24%) and Amikacin (14%). On the other hand, 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.

Twenty IRAB isolates (91.3%) showed biofilm-forming ability. Strong biofilm producers (80.95 %) were the most common biofilm pattern observed among IRAB while the remaining isolates showed moderate and weak biofilm forming ability (9.52 % for each) (Fig. 1).

Metallo-β-lactamase (MBL) production was phenotypically detected by CDT. Out of 23 of IRAB 19 isolates (82.6%) were MBL producers using CDT (Fig. 2).

Among the 19 CDT-positive *A. baumannii* isolates, 11 (57.9%) isolates harboured MBL-encoding gene (*bla*NDM). PCR revealed amplification of a 129 bp fragment corresponding to the *bla*NDM gene, and no isolates showing the presence of other tested MBL genes (Fig. 3).

Referring to the association between the antibiotic resistance and production of MBLs in *A. baumannii,* there was a statistically significant relationship between MBLs production and resistance against ciprofloxacin, sulfamethoxazole-trimethoprim, imipenem, gentamycin, and amikacin (Table. 3).

In this study, a total of 23 IRAB 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 the tested isolates were positive for *adeB* gene as they showed the expected amplicon of 541 bp (Fig. 4).

In the present study, a total of 19 MBL producing *A. baumannii* isolates were investigated for the presence of class 1 integron using conventional PCR and specific primer for class 1 integron. All isolates showed positive class 1 integron gene (Fig. 5)

Table 1. Classification of biofilm formation potential.

Biofilm formation	**Optical density (OD)**
Non-adherent	**OD**≤ODC
Weak	ODC**<OD≤(**2×ODC)
Moderate	(2×ODC**) <OD≤(**4×ODC)
High	(4×ODC) <**OD**

ODC (Optical Density Cut Off) =(SD×3) above the mean optical density of control.

Table 2. List of multiplex PCR primers:

Target(s)	**Primer**	**Sequence**	**Expected amplicon size**	**References**
bla_{VIM}	*VIM -F*	5'- GATGGTGTTTGGTCGCATA-3'	390 bp	**(Dallenne *et al.*, 2010)**
	VIM -R	5'-CGAATGCGCAGCACCAG-3'		
bla_{IMP}	*IMP-F*	5'-TTGACACTCCATTTACDG-3'	139 bp	**(Dallenne *et al.*, 2010)**
	IMP -R	5'-GATYGAGAATTAAGCCACYCT-3'		
bla_{NDM}	*NDM- F*	5'-CCCGGCCACACCAGTGACA-3'	129 bp	**(Voets *et al.*, 2011)**
	NDM -R	5'- GTAGTGCTCAGTGTCGGCAT-3'		
bla_{SIM}	*SIM -F*	5'- TTGCGGAAGAAGCCCAGCCAG-3'	613 bp	**(Voets *et al.*, 2011)**
	SIM -R	5'-GCGTCTCCGATTTCACTGTGG-3'		
bla_{GIM}	*GIM-F*	5'-CGTTGCCAGCTTTAGCTCAGG-3'	279 bp	**(Voets *et al.*, 2011)**
	GIM- R	5'-GCAACTTGATACCAGCAGTGCG-3'		
bla_{SPM}	*SPM -F*	5'- GGGTGG CTAAGACTATGAAGCC-3'	447 bp	**(Voets *et al.*, 2011)**
	SPM - R	5'-GCCGCCGAGCTGAATCGG-3'		

Table 3. Relationship between antibiotic resistance and MBLs production in *A. baumannii* isolates.

Antimicrobial agents	**MBL (n= 19)**		**Non-MBL (n=40)**		***P-value***
	R	S	R	S	
Piperacillin	19	0	34	6	0.1632
Piperacillin-tazobactam	19	0	34	6	0.1632
Levofloxacin	19	0	34	6	0.1632
Ciprofloxacin	19	0	31	9	**0.0465**
Sulfamethoxazole-trimethoprim	19	0	31	9	**0.0465**
Ceftazidime	19	0	35	5	0.1647
Ceftriaxone	19	0	34	6	0.1632
Imipenem	19	0	18	22	**0.0002**
Gentamycin	19	0	26	14	**0.0024**
Amikacin	18	1	26	14	**0.0227**
Ampicillin-Sulbactam	19	0	35	5	0.1647

Figure 1. Frequency of biofilm forming isolates of IRAB.

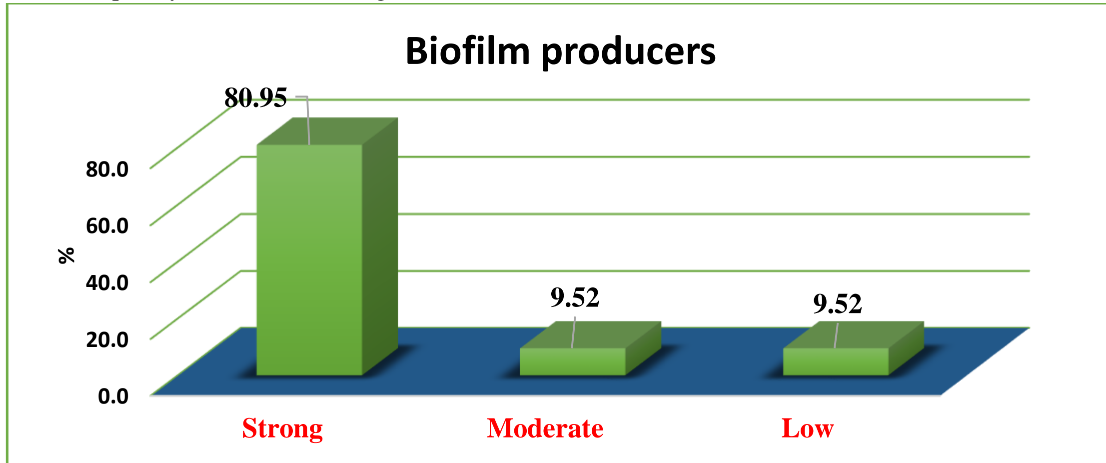


Figure 2. Positive combined disc test for detection of MBL producer. The test isolate shows a zone diameter>7mm around the imipenem-EDTA disc compared to that of the imipenem disc alone.

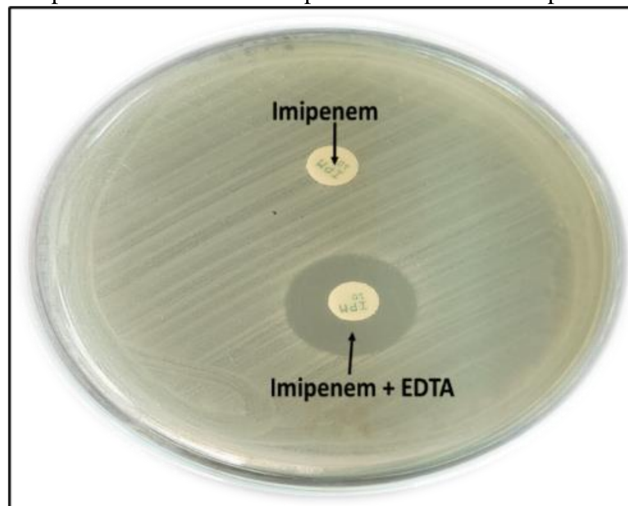


Figure. 3. Agarose gel electrophoresis of *blaNDM* gene amplified from 19 CDT-positive *A. baumannii* isolates using 100 bp DNA marker. N: negative control, L: ladder

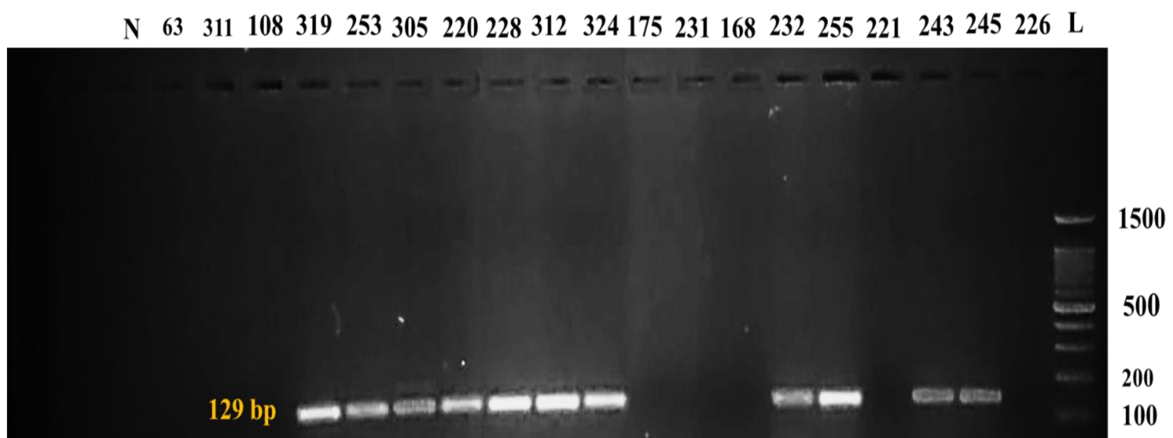
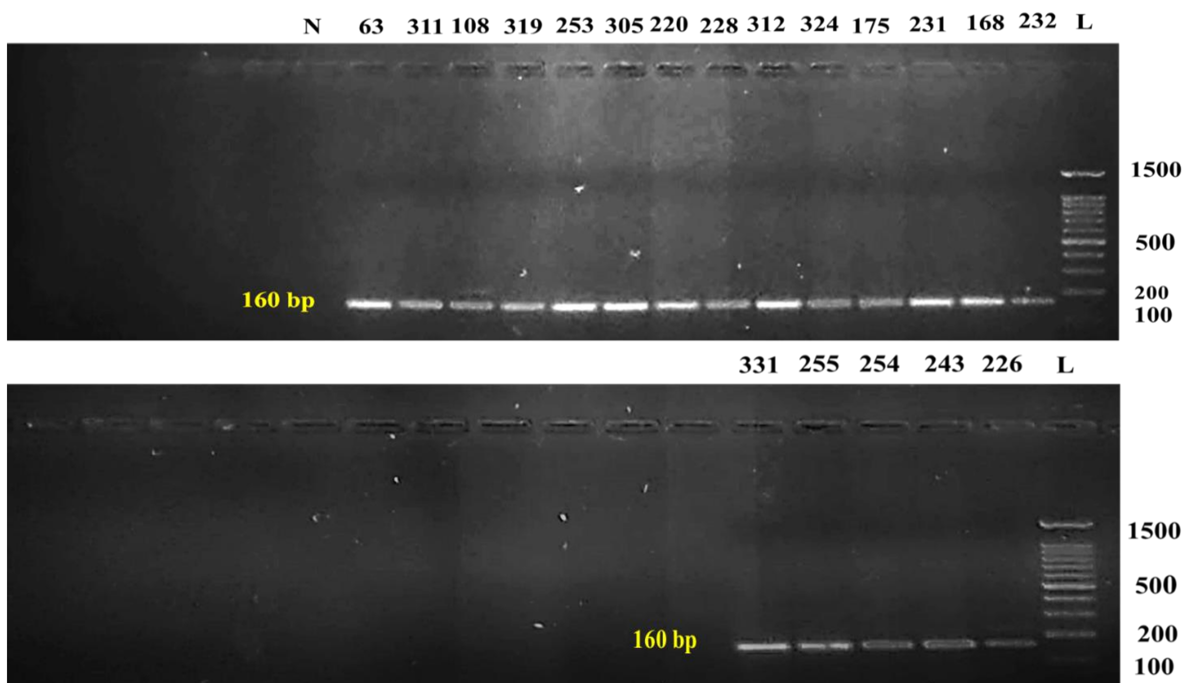


Figure 4. Agarose gel electrophoresis of *adeB* gene amplified from 23 Imipenem-resistant *A. baumannii* isolates using 100 bp DNA marker. N: negative control

Figure 5. Agarose gel electrophoresis of Class 1 integron gene amplified from 19 MBL producing *A. baumannii* isolates using 100 bp DNA marker. N: negative control, L: ladder



Discussion

In this study, 23 (38.98%) *A. baumannii* isolates exhibited resistance to imipenem. results were consistent with previous research from Egypt [16,17], which reported imipenem resistance rates among *Acinetobacter* species of 25% and 20%, respectively. Additionally, an Indian study found that 23% of *Acinetobacter* species were imipenem resistant [11]. Higher resistance rates 88% and 91.5%, respectively were reported in Iran and China [18,19]. The rise in carbapenem-resistant *A. baumannii* may be due to the extensive misuse of carbapenems and poor adherence to infection control guidelines.

Metallo-β-lactamases are undermining the effectiveness of β-lactam antibiotics, especially carbapenems, which are used as a last-resort antibiotics indicated for a variety of multidrug-resistant bacterial infections [11]. Researchers have used a variety of molecular and non-molecular methods to detect MBL production in bacterial isolates [19]. In the present study, 19 out of 23 imipenem-resistant *A. baumannii* isolates (82.6%) were identified as MBL producers using the CDT. This finding was in accordance with previous research [20], which reported that 80% of carbapenem resistant *A. baumannii* isolates collected from Egyptian patients were MBL producers. Similar results were obtained in other studies [21,22], with 86.8% and 81.18% of isolates recognized as MBL producers by CDT. The high rate of MBL expression in *A. baumannii* isolates demonstrates that it has become a significant contributing factor to imipenem resistance among these species.

Molecular-based techniques continue to be the golden method to confirm the presence of MBL genes [23]. In the current study, multiplex PCR analysis revealed amplification of a 129 bp fragment corresponding to the bla_{NDM} gene in 11 out of 19 (57.9%) CDT-positive *A. baumannii* isolates, while no other MBL genes were detected. This outcome was consistent with previous studies in Egypt that reported a high incidence and predominance of bla_{NDM} gene in *A. baumannii* and other Gram-negative bacterial isolates recovered from hospitalized patients, with bla_{NDM} gene found in 68.2%, 70.0%, and 88.4% of isolates, respectively, compared to other genes, revealing the emergence and rapid spread of this gene across different regions in Egypt [20,24,25].

Phenotypically MBLs producing isolates by CDT with negative PCR results could be explained by the fact that these isolates might harbour different variants of the studied genes or additional MBL genes not covered in our study. Imipenem-resistant isolates that did not show MBL activity through CDT might harbor other carbapenemases, such as OXA-type (class D) or AmpC (class C) β-lactamases, and/or other carbapenem resistance mechanisms such as mutations of porin-encoding genes, overexpression of efflux pump genes and/or outer-membrane permeability mechanisms, which were not investigated in our study.

The widespread of MBL genes across various organisms indicates a potential for significant genetic transfer. Several studies have reported that that MBLs are carried on gene cassettes inserted into class 1 integrons in clinical isolates of *Pseudomonas* spp., and *Enterobacteriaceae* [26]. In the current study all *A. baumannii* isolates that had bla_{NDM} gene were also class 1 integron carriers suggesting that class 1 integron plays a major role in horizontal gene transfer in increasing the dissemination of bla_{NDM} gene in *A. baumannii* isolates.

In this study, twenty-one IRAB isolates (91.3%) showed biofilm-forming ability, of which 80.9% were MBLs producers and all of biofilm forming isolates were MDR. There was a statistically significant relationship between MBLs production and resistance against ciprofloxacin, sulfamethoxazole-trimethoprim, imipenem, gentamycin, and amikacin. Our findings demonstrate a high prevalence of biofilm-forming and MBL production in multidrug-resistant organisms in our institution, which displays the significant role of biofilm and MBL production in conferring antibiotic resistance and provides a glimpse of an upcoming threat in our hospitals.

The AdeABC efflux pump has been shown to mediate carbapenem resistance in clinical isolates of *Acinetobacter baumannii*, as demonstrated in a previous study, that investigated the correlation between overexpression of the *adeB* gene and increased resistance levels [27]. In this study, a total of 23 Imipenem-resistant *A. baumannii* isolates were investigated for the presence of AdeABC efflux pump genes by PCR. 18 out of 23 (78.3%) isolates were positive for *adeB* gene. This finding agreed with previous researchers by [28,29], who reported even higher detection rates 94% and 100%, respectively. These results strongly suggest the role of efflux pumps in carbapenem resistance in *A. baumannii*. However, however further research for assessing *adeB* expression is required to clarify this relationship.

This study had some limitations, including a relatively small sample size and the identification of isolates based solely on phenotypic methods, detection of biofilm formation using single phenotypic method without confirmation with molecular method. Additionally, further investigation into the role of the AdeABC efflux pump in carbapenem resistance using RT-PCR is

needed to confirm the findings from conventional PCR.

Conclusion

Our investigation underscores the alarming emergence and dissemination of *bla*NDM gene in our hospitals. The capacity of these bacteria to form biofilms and produce MBL enzymes exacerbates this public health crisis. Notably, all isolates harbouring MBL genes also contained class 1 integrons, emphasizing the crucial role of these genetic elements in facilitating the spread of antibiotic resistance.

Declarations

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

All datasets generated or analyzed during this study are included in the manuscript.

Competing interests

There is no conflict of interest.

Authors' contribution

All authors contributed substantially to the intellectual content of this study, and approved it for publication.

Funding:

None.

Acknowledgments:

None

References

1- Shi X, Wang H, Wang X, Jing H, Duan R, Qin S, Wang J. Molecular characterization and antibiotic resistance of Acinetobacter baumannii in cerebrospinal fluid and blood. PLOS One. 2021;16: 1-13.
2- Benmahmod AB, Said HS, Ibrahim RH. Prevalence and mechanisms of carbapenem resistance among Acinetobacter baumannii clinical isolates in Egypt. Microb Drug Resist. 2019;25: 480-488.
3- Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant Acinetobacter baumannii isolates from Egypt. Antimicrob Resist Infect Control. 2019;8: 1-9.
4- Lin L, Ling BD, Li XZ. Distribution of the multidrug efflux pump genes adeABC, adeDE, and adeIJK and class 1 integron genes in multiple-antimicrobial-resistant clinical isolates of Acinetobacter baumannii–Acinetobacter calcoaceticus complex. Int J Antimicrob Agents. 2009;33: 27-32.
5- Colquhoun JM, Rather PN. Insights into mechanisms of biofilm formation in Acinetobacter baumannii and implications for uropathogenesis. Front Cell Infect Microbiol. 2020;10: 1-18.
6- Baniya B, Pant ND, Neupane S, Khatiwada S, Yadav UN, Bhandari N, Chaudhary R. Biofilm and metallo beta-lactamase production among the strains of Pseudomonas aeruginosa and Acinetobacter spp., at a tertiary care hospital in Kathmandu, Nepal. Ann Clin Microbiol Antimicrob. 2017;16: 1-4.
7- Khodier AA, Saafan A, Bakee W, Khairalla AS. Molecular characterization of multiple antibiotic-resistant Acinetobacter baumannii isolated from Egyptian patients. J Pure Appl Microbiol. 2020;14: 2399-2405.
8- Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Patho. 1966;45: 493-496.
9- Clinical and Laboratory Standards Institute (CLSI). Performance standard for antimicrobial susceptibility testing; twenty-seventh informational supplement. M100-S26. 2017;37(1).
10- Amoli RI, Nowroozi J, Sabokbar A, Rajabniya R. Isolation of Stenotrophomonas maltophilia from clinical samples: An investigation of patterns motility and production of melanin pigment. Asian Pac J Trop Biomed. 2017;7: 826-830.

11- Solanki S, Saileela K, Dinesh V, Kumar S, Trinain Khant N, Purti. Detection of metallo beta lactamase production in imipenem-resistant Gram-negative nonfermenting bacilli isolated in a tertiary care hospital in south India. Int J Contemp Med Res. 2020;7: 11-14.

12- Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother. 2010;65: 490-495.

13- Voets GM, Fluit AC, Scharringa J, Stuart JC, Leverstein-van Hall MA. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. Int J Antimicrob Agents. 2011;37: 356-359.

14- Adwan K. Fast DNA isolation and PCR protocols for detection of methicillin-resistant staphylococci. Folia Microbiol. 2014;59: 5-8.

15- Shaye MA, Sharifmoghadam MR, Bahreini M., Ghazvini K. Mafinezhad A. Amiri G. Study of the role of efflux pumps in amikacin-resistant Acinetobacter isolates from teaching hospitals of Mashhad, Iran. Jundishapur J Microbiol. 2018;11: 1-7.

16- Marei YE, Al-Sweify MA, Mansour MK, Kishk RM. Prevalence and antimicrobial susceptibility patterns of non-fermenting Gram-negative bacilli in Ismailia, Egypt. Egypt J Med Microbiol. 2019;28: 121-125.

17- Abd El-Baky RM, Farhan SM, Ibrahim RA, Mahran KM, Hetta HF. Antimicrobial resistance pattern and molecular epidemiology of ESBL and MBL producing Acinetobacter baumannii isolated from hospitals in Minia, Egypt. Alexandria J Med. 2020;56: 4-13.

18- Hashemi B, Afkhami H, Khaledi M, Kiani M, Bialvaei AZ, Fathi J, Ahanjan M. Frequency of Metalo beta Lactamase genes, blaIMP1, INT 1 in Acinetobacter baumannii isolated from burn patients North of Iran. Gene Rep. 2020;21: 1-5.

19- Mohamed NM, Raafat D. Phenotypic and genotypic detection of metallo-beta-lactamases in imipenem-resistant Acinetobacter baumannii isolated from a tertiary hospital in Alexandria, Egypt. Res J Microbiol. 2011;6: 750-760.

20- Abo-Alella D, Malek MM. Phenotypic and genotypic characterization of metallo beta lactamases among Gram-negative uropathogens from a tertiary care hospital in Egypt: a cross sectional study. Microbes Infect Dis. 2021;2: 295-307.

21- Noori M, Karimi A, Fallah F, Hashemi A, Alimehr S, Goudarzi H, Aghamohammad S. High prevalence of metallo-beta-lactamase producing Acinetobacter baumannii isolated from two hospitals of Tehran, Iran. Arch Pediatr Infect Dis. 2014;2: 1-5.

22- Shivaprasad A, Antony B, Shenoy P. Comparative evaluation of four phenotypic tests for detection of metallo-β-lactamase and carbapenemase production in Acinetobacter baumannii. J Clin Diagn Res. 2014;8: DC05–DC08.

23- Farrag H, El-Shatoury E, El-Baghdady K, Nada H, Fawkia M. Prevalence of metallo-β-lactamase genes in multidrug-resistant Gram-negative bacilli. Egypt J Exp Biol. 2015;11: 63-69.

24- El-Domany RA, El-Banna T, Sonbol F, Abu-Sayedahmed SH. Co-existence of NDM-1 and OXA-48 genes in carbapenem-resistant Klebsiella pneumoniae clinical isolates in Kafrelsheikh, Egypt. Afr Health Sci. 2021;21: 489-496.

25- Elbadawi HS, Elhag KM, Mahgoub E, Altayb HN, Ntoumi F, Elton L, McHugh TD. Tembo J, et al. Detection and characterization of carbapenem-resistant Gram-negative bacilli isolates recovered from hospitalized patients at Soba University Hospital, Sudan. BMC Microbiol. 2021;21: 1-9.
26- Farrag H, El-Shatoury E, El-Baghdady K, Nada H, Fawkia M. Prevalence of metallo-β-lactamase genes in multidrug resistant Gram-negative bacilli. Egypt J Exp Biol. 2015;11: 63-9.
27- Lee Y, Yum JH, Kim CK, Yong D, Jeon EH, Jeong SH, Ahn JY, Lee K. Role of OXA-23 and AdeABC efflux pump for acquiring carbapenem resistance in an Acinetobacter baumannii strain carrying the blaOXA-66 gene. Ann Clin Lab Sci. 2010;40: 43-48.
28- Hou PF, Chen XY, Yan GF, Wang YP, Ying CM. Study of the correlation of Imipenem resistance with efflux pumps AdeABC, AdeIJK, AdeDE and AbeM in clinical isolates of Acinetobacter baumannii. Chemotherapy. 2012;58: 152-158.
29- JaponiNejad AR, Sofian M. Ghaznavi-Rad E. Molecular detection of AdeABC efflux pump genes in clinical isolates of Acinetobacter baumannii and their contribution in imipenem resistance. ISMJ. 2014;17: 815-823.

Gaballah M, Hefny H, Mansy, M, Hassan, RM. Dissemination of NDM-mediated carbapenem resistance in biofilm-forming Acinetobacter baumannii in some Egyptian hospitals. Microbes Infect Dis 2024; 5(4): 1461-1470.