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Genotyping of carbapenem resistant *Acinetobacter baumannii* isolated from Egyptian patients

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Abstract

Acinetobacter baumannii has recently been known as a major cause of hospital- and community-acquired infections. Carbapenem resistant *A. baumanni* (CRAB) has been recorded to be resistant to nearly all antibiotics, including the last resort antibiotics; carbapenems. This study aimed to detect the carbapenem resistance levels and mechanisms, in addition to the genotyping of *A. baumanni* in Upper Egypt. About 200 clinical samples were collected from different wards of Sohag University Hospital, Egypt, from which 20 *A. baumannii* isolates were recovered and then identified using conventional methods and Polymerase Chain Reaction (PCR). Antibiotic sensitivity testing was carried out using the Disk diffusion method, followed by PCR testing of the common carbapenemase-encoding genes, including *OXA-51*, *OXA-58*, *KPC*, *GES*, *IMP*, *NDM*, *VIM*, *SIM*, and *GIM*. Genotyping was performed using the Enterobacterial Repetitive Intergenic Consensus-Polymerase chain reaction (ERIC-PCR). About 85 % of *A. baumannii* strains were multidrug resistant (MDR), and high rate of Extreme drug resistant (XDR) *A. baumannii* (70 %) was detected. Carbapenem resistance was detected in 65 % of *A. baumannii* isolates, 70.58 % of MDR isolates, and 85.7 % of XDR isolates, respectively. Carbapenemase- encoding genes, including bla$_{OXA-51}$, *VIM*, *NDM*, and *GES*, were detected in 100 %, 100 %, 76.9 2 % and 76.92 % of the carbapenem resistant *A. baumannii* (CRAB) isolates, respectively. The bla$_{IMP}$ and bla$_{KPC}$ genes had lower prevalence rates of 15.38 % and 30.77 %; respectively, whereas the *SIM*, *GIM*, and *OXA-58* genes were not detected in any of the tested *A. baumanni* isolates. All of the MDR isolates carried three or more the carbapenemases encoding genes, and 85.7 % of the XDR isolates carried four or more of the carbapenemase-encoding genes. The dendrogram

constructed from the ERIC-PCR results showed that the *A. baumannii* isolates were divided into three different clusters.

Keywords: *Acinetobacter baumannii*, MDR, CRAB, ERIC-PCR, Carbapenemases

1. Introduction

Antimicrobial resistance is a major public health concern, as it limits the infection treatment options (Park *et al*., 2019). *A. baumannii*, is a serious endemic and widespread bacterial pathogen that causes nosocomial infections, and is at the forefront of this serious problem (Ridha *et al*., 2019). This bacterium is an aerobic non-fermentative Gram-negative bacilli that is ranked as second after *Pseudomonas aeruginosa* (Talukdar *et al*., 2018). Moreover, *A. baumannii* is considered as a member of the ESKAPE pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, and Enterobacter spp., which are group of pathogens that have become resistant to multiple antibiotic classes (Ranu *et al*., 2019).

The endemic and epidemic behavior of this opportunistic pathogen may be attributed to its ability to endure the harsh hospital conditions for extended periods of time (Namiganda *et al*., 2019). Additionally, the pathogen's persistent presence in the hospitals exposes it to several antibiotics, which places the pathogens that have antibiotic resistance under a selective pressure particularly in the intensive care units (ICUs). *A. baumannii* regularly attacks the most vulnerable hospital patients.

A previous study conducted by Chen *et al*., (2019) revealed that pneumonia is the most frequent clinical manifestation of *A. baumannii* infections, which is listed as the 5th most prevalent infection in ICUs of both the developed and developing countries (Hassannejad *et al*., 2019). Furthermore, the existence of numerous resistance mechanisms in this strain and its capacity to acquire new resistance traits against the current antibiotics facilitates the development of nosocomial infections.

The antibacterial resistance arsenals of *A. baumannii* include enzymatic inactivation of the antibiotics; target membrane alterations, and active drug export via membrane-localized drug efflux transporters (Coskun *et al*., 2019). The MDR *A. baumannii* strains (MDR-Ab) are those that show resistance to 3 or more different antibacterial classes, whereas those strains that are resistant to all but 2 drug classes are referred to as Extreme drug resistant *A. baumannii* (XDR-Ab). A previous study of Smiline and Vijayashree, (2019) revealed that there has been an increase in the number of *A. baumannii* isolates that are resistant to carbapenems; colistin, and polymyxins, as well as other drug classes, a condition that is known as pan-drug resistance (PDR). Carbapenems are the last option antibiotics for treating these MDR pathogens; however, recent findings indicate that resistance to these antibiotics is also rising (Mohammed and Singh, 2019). Development of β-lactamases; changes in cell membrane permeability, and increased expression of the efflux genes are some of the mechanisms that cause carbapenem resistance in *A. baumannii* (Dagher *et al*., 2019). Based on the molecular ambler classification, these enzymes have been categorized as existing in classes A, B, and D. The Guiana extended spectrum β-lactamase (GES) is most prevalent in *A. baumannii*, which confers a low level of resistance to carbapenems. Class D β-lactamases are prevalent in *A. baumannii*, which are also known as *OXA*-type enzymes or oxacillinases, as highlighted by Chen *et al*., (2019).

Tetracyclines, including doxycycline and minocycline, as well as other glycylcyclines are additional options for treatment of infections caused by the carbapenems resistant *A. baumannii* strains (CRAB). Doxycycline and minocycline are

particularly efficient classes of antibiotics against the ventilator-associated pneumonia (VAP) that is caused by these carbapenems resistant strains (Nguyen and Joshi, 2021). In case of infection by XDR-Ab or CRAB, colistin should be used in combination with several antibiotics, including carbapenems; rifampin, piperacillin-tazobactam, and sulbactam (Quoc *et al*., 2019).

The objectives of this study were to evaluate the antibiotic resistance patterns, and to detect frequency of the most common carbapenemase genes in *A. baumannii* isolates, which were recovered from patients attending to the Sohag University Hospital in Upper Egypt, in addition to detecting the genetic relatedness among these collected isolates.

2. Materials and methods

2.1. Samples collection

This is a cross-sectional study that is conducted in Microbiology and Immunology Department and the Central Research Laboratory, Sohag University Hospital, Egypt, during the period from February, 2020 to September, 2021. Clinical data of the study participants were collected from their medical records, including the age, sex, type of infection, associated comorbidities such as hypertension and diabetes, presence of external device, history of trauma, burn, cancer and\ or surgical intervention. About 200 clinical samples were collected from patients admitted to the different hospital wards, including chest; neurology, plastic surgery, general surgery, oncology, and vascular surgery. The different samples had been collected from patients that had several types of infections, such as pus collected from surgical site infection "SSI", infected burn, abscess, diabetic foot, diabetic abscess and chronic ulcer, sputum obtained from patients with pneumonia, endotracheal aspirate collected from patients with VAP, and cerebrospinal fluid collected from patients with meningitis. All age groups were included in the study (3-80 years). Informed consents were taken from all the patients or their relatives.

2.2. Isolation and identification of the *Acinetobacter* sp.

Clinical samples were transported to the microbiology laboratory and were inoculated directly on MacConkey agar plate (Oxoid, UK), and then incubated at 37ºC. After 24 h of incubation, the growing pale bacterial colonies were streaked on CHROMagar TM *Acinetobacter* (CHROMagar, Paris, France), which is a selective and differential chromogenic culture medium used for isolation and identification of *Acinetobacter*. The developing *Acinetobacter* colonies were purified and examined microscopically after staining by Gram stain, and were tested biochemically using oxidase test. Confirmation of identification of the *Acinetobacter* isolates was carried out molecularly by tracking the bla_{OXA-51} gene (El-Badawy *et al*., 2019). All the *Acinetobacter* isolates were stored in soya broth at -80 ºC till further processing.

2.3. Antibiotic sensitivity assay

According to the Clinical and Laboratory Standards Institute, 2019 (Smiline and Vijayashree, 2019), an antibiotic sensitivity test was performed for 20 *A. baumannii* isolates by disc diffusion assay. The surface of Muller Hinton agar (Oxoid-UK) plates were swabbed using a sterile cotton swab, which had been dipped in a bacterial suspension adjusted to 0.5 McFarland standard turbidity. Several antibiotic discs were placed individually on the seeded plate surface, including piperacillin; ampicillin-sulbactam, piperacillin-tazobactam, doxycycline, minocycline, ciprofloxacin, levofloxacin, trimethoprim-sulphamethoxazole, gentamicin, amikacin, imipenem, meropenem, ceftazidime, and ceftriaxone (Bioanalyse, Ankara, Turkey). After incubation at 37^{o}C for 24 h, the diameter of the developing inhibition zone was measured using a calibrated ruler. The assay was carried out in triplicates for each tested antibiotic. Results were interpreted into three categories; mainly resistant, intermediate, and sensitive, according to Smiline and Vijayashree, (2019).

2.4. Molecular characterization of *Acinetobacter* sp.

2.4.1. DNA extraction

The bacterial deoxynucleic acid (DNA) was extracted by the Boiling method of Chen *et al*., (2020). About 3-5 colonies of each *Acinetobacter* isolate were emulsified in 100 µl of sterile dist. water, incubated at 95 °C on a heat block (Thermo Fisher Scientific, UK) for 15 min., and centrifuged at 10.000 rpm for 10 min. The extracted DNA was stored at -20°C for amplification of the target genes using PCR.

2.4.2. Amplification of the extracted DNA

Molecular detection of *A. baumannii* isolates was carried out by PCR using specific primers (Table 1), to amplify the bla$_{OXA-51}$-like gene, and to molecularly characterize the carbapenemase encoding genes that belong to Ambler classes A, B, and D, including *OXA-58*, *VIM*, *SIM*, *GIM*, *IMP*, *NDM*, *KPC*, and *GES*. PCR reaction was carried out in a final volume of 25 µl, including 5 µl of the extracted DNA, 1.25 µl of each primer (Metabion, Germany), 12.5 µl of master mix (Willowfort, UK), and 5 µl of sterile dist. water. DNA amplification was performed using a thermal cycler (T Gradient-Biometra, Germany). The cyclic conditions of the PCR reaction were programed as follow: one cycle of initial denaturation at 94 °C for 5 min., followed by 35 cycles consisting of 3 steps, mainly DNA denaturation at 94 °C for 30 sec, annealing temperatures were adjusted according to the melting temperature of each primer (Tm-5) for 30 sec, and elongation at 72 °C for 1 min.\1 Kb, followed by a final elongation at 72 °C for 10 min. The reaction was terminated by cooling at 4°C, in reference to Wasfi *et al*., (2021).

The amplified products were separated on 2 % agarose gel that was then stained with Ethidium bromide. The stained gels were visualized, documented with a gel documentation system (InGenius 3, UK), and analyzed visually to determine size of the PCR amplicons of the target genes directly, compared with the DNA ladder (Willowfort BERUS 100bp, WF-WF10407001, UK).

2.5. Molecular typing of Enterobacterial Repetitive Intergenic Consensus-PCR

Clonal relatedness of *A. baumannii* was examined via Enterobacterial Repetitive Intergenic Consensus-Polymerase chain reaction (ERIC)-PCR using the following primers; ERIC1 5´-GTGAATCCCCAG-GAGCTTACAT-3′ and ERIC2 5´- AAGTAAGTGACT-GGG-GTGAGCG-3′. The specificity of these primers was checked by The National Center for Biotechnology Information (NCBI)-Blast of the GenBank database. The PCR reaction composed of 10 µl of Master Mix (Willowfort, UK), 0.5 µl of the extracted DNA, 1 µl of each forward and reverse primers (10 Pmol) (Metabion, Germany), and 7.5 µl of distilled water, in a total PCR reaction volume of 20 µl. The cyclic conditions for ERIC-PCR included; initial denaturation at 94 °C for 5 min., 40 cycles including 94 °C for 5 min., 94 °C for 1 min., annealing step at 52 °C for 2 min., elongation step at 72 °C for 2 min., and final elongation at 72 °C for 5 min. (Zarifi *et al*., 2020). The amplified products were visualized on 2 % agarose gel stained with Ethidium bromide. The stained gels were visualized and documented with a gel documentation system (InGenius 3, UK).

2.6. Data analysis

The agarose gel photos were scanned by the Gene Profiler 4.03 computer software program that uses automatic lane and peak finding for detecting the presence of banding patterns, and then calibrating them for size and intensity. A binary data matrix recording the presence (1) or the absence (0) of bands was made. The software package MVSP (Multi-Variate Statistical Package) was used and genetic similarities were computed using the Dice coefficient of similarity of Nei and Li (Ranjbar *et al*., 2017). Isolates with more than 90 % similarity were considered to belong to the same ERIC cluster. Cluster analysis was carried out on similarity estimates using

Table 1: Sequence of primers used to identify *A. baumannii* isolates and to detect the carbapenemase encoding genes

Genes	Melting temperature	Nucleotide sequence (5′ → 3′)	Amplicon size (bps)	Reference
OXA-51	56 °C 57 °C	TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTTGG	353	(Al Amri *et al*., 2020)
OXA-58	58 °C 63 °C	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	599	(Abouelfetouh *et al*., 2019)
VIM	55 °C 57 °C	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390	(Al Amri et al., 2020)
SIM	57 °C 58 °C	TACAAGGGATTCGGCATCG TAATGGCCTGTTCCCATGTG	571	(Wasfi *et al*., 2021)
GIM	58 °C 56 °C	TCGACACACCTTGGTCTGAA AACTTCCAACTTTGCCATGC	477	(Abouelfetouh *et al*., 2019)
IMP	56 °C 56 °C	CATGGTTTGGTGGTTCTTGT ATAATTTGGCGGACTTTGGC	188	(Shirmohammadlou *et al*., 2018)
NDM	60 °C 56 °C	GCAGGTTGATCTCCTGCTTG ACGGTTTGGCGATCTGGT	203	(Al Amri *et al*., 2020)

KPC	58 °C 60 °C	ATCTGACAACAGGCATGACG GACGGCCAACACAATAGGTG	452	(Al Amri *et al*., 2020)
GES	57 °C 59 °C	GATACAACTACGCCTATTGCT CAGCCACCTCTCAATGGTG	99	(Wasfi *et al*., 2021)

the un-weighted pair-group method with arithmetic average (UPGMA) software. These methods were carried out using MVSP software programs.

2.7. Statistical analysis

Data was analyzed using STATA version 14.2 (Stata Statistical Software: Release 14.2 College Station, TX: StataCorp LP.). As the data was not normally distributed, the Mann-Whitney test was used to compare between two groups. The qualitative data was presented as numbers and percentages, and then compared using either Chi square test or fisher exact test. The odds ratios were obtained from the logistic regression analysis. Graphs were produced by using Excel program. The *p*-value was considered significant if it was less than 0.05.

3. Results

3.1. Isolation and characterization of the *A. baumannii* isolates

In this study, the *Acinetobacter* isolates were Gram-negative coccobacilli, oxidase negative, and gave pale non-lactose fermenting colonies on MacConkey agar, and red colonies on CHROMagar TM *Acinetobacter* medium. About 20 *A. baumannii* isolates were recovered from different wards of Sohag University Hospital, including vascular surgery (25 %), ICU (20 %), chest department (15 %), general surgery (15 %), plastic surgery (10 %), oncology (10 %), and neurosurgery (5 %), and from several types of infections; mainly VAP (20 %), surgical site infection "SSI" (20 %), pneumonia (15 %), diabetic foot (15 %), infected burn (10 %), abscess (10 %), chronic ulcer (5 %), and from meningitis (5 %). Identification of *A. baumannii* was confirmed through detecting the bla_{OXA-51} gene, which was recorded in 95 % of the isolates. The demographic data revealed that the mean age of patients included in this study was 43.7± 21.77 years, and 60 % of *A. baumannii* isolates were recovered from male patients. Furthermore, the most important risk factors associated with *A. baumannii* infections were surgery (40 %), followed by chronic debilitating diseases such as diabetes (30 %), in addition to the presence of external devices (20 %).

3.2. Antibiotic susceptibility patterns of *A. baumannii* isolates

Results of the antibiotic susceptibility patterns are demonstrated in Fig. (1). The lowest recorded resistance rates for both minocycline and doxycycline were 15 % and 10 %, respectively. Carbapenem resistance was detected in 65 % of *A. baumannii* (CRAB), and 35 % of the isolates were sensitive to carbapenems (carbapenem sensitive *A. baumannii*), as shown in Table (2). About 85 % of the *A. baumannii* isolates were categorized as MDR, based on resistance to at least 1 antibiotic in ≥ 3 antibiotic group.

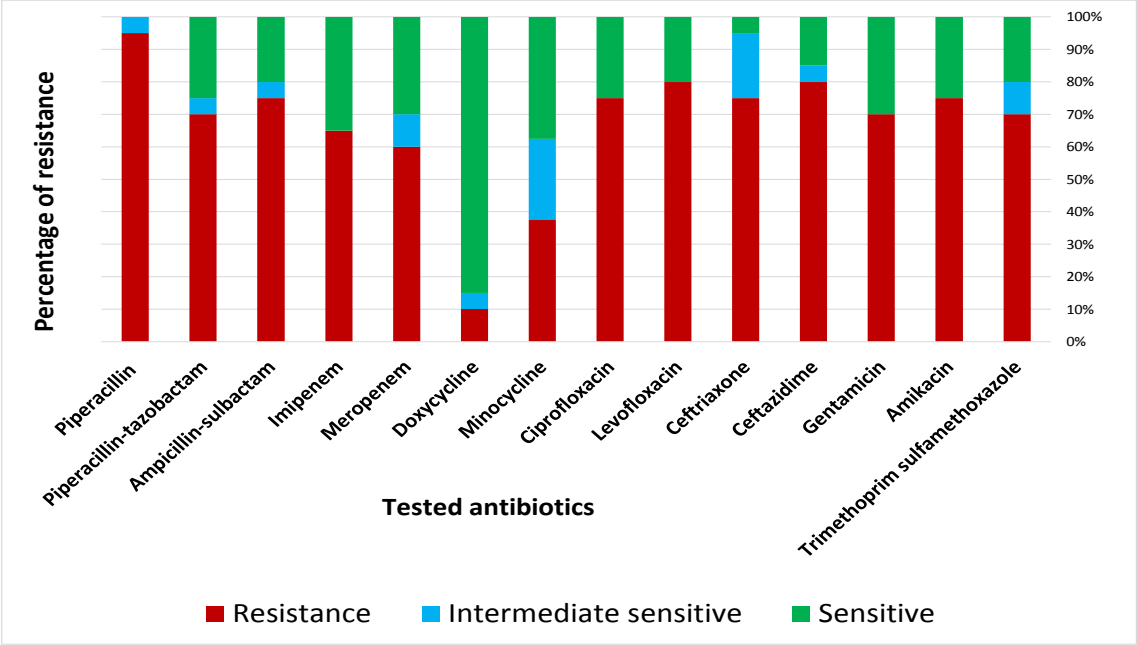

Fig. 1: Percentages of interpretive resistance categories (i.e., resistant, intermediate, and sensitive) of 20 *A. baumannii* isolates to the different antibacterial agents. Results are averages of 3 replicates for each tested antibiotic

Table 2: Frequency and percentages of MDR, XDR, CRAB, and ERIC groups among *A. baumannii* isolates

Variable	**Number and percentage (%) of *A. baumannii* isolates**
MDR *A. baumannii*	
No	3 (15.00 %)
Yes	17 (85.00 %)
XDR *A. baumannii*	
No	6 (30.00 %)
Yes	14 (70.00 %)
CSAB	7 (35.00 %)
CRAB	13 (65.00 %)

ERIC groups	
A	4 (20.00 %)
B	1 (5.00 %)
C1	3 (15.00 %)
C2	12 (60.00 %)

Where: MDR: Multidrug resistant, XDR: High rate of extreme drug resistant, ERIC: Enterobacterial repetitive intergenic consensus

3.3. Detection of carbapenemase genes

The PCR was used to detect the carbapenemase-encoding genes; mainly Ambler class A (*KPC* and *GES*), Ambler class B (*IMP*, *VIM*, *GIM*, *NDM*, and *SIM*), and Ambler class D (*OXA51* and *OXA58*). The carbapenemase-encoding resistance genes *VIM*, *OXA-51*, *NDM*, and *GES*, were detected with the highest prevalence of 95 %, 95 %, 85 %, and 80 % in *A. baumannii* isolates, respectively. However, the *OXA58*, *SIM*, and *GIM*, were not detected in any isolate (Table 3).

3.4. Correlation analysis between AST phenotypes and carbapenem resistance genes

Results presented in Table (4) show that none of the tested resistance genes had significant statistical association with the carbapenem resistance phenotypes, as both of the CRAB and CSAB were found to carry carbapenem-hydrolyzing genes with minimal differences.

Table 3: Distribution of the carbapenem resistance genes among *A. baumannii* isolates `

Ambler class	Resistance genes	Negative n (%)	Positive n (%)
A	*GES*	4 (20.00 %)	16 (80.00 %)
	KPC	12 (60.00 %)	8 (40.00 %)
B	*SIM*	20 (100 %)	0
	GIM	20 (100 %)	0
	NDM	3 (15.00 %)	17 (85.00 %)
	VIM	1 (5.00 %)	19 (95.00 %)
	IMP	17 (85.00 %)	3 (15.00%)

	OXA51	1 (5.00 %)	19 (95.00 %)
D	*OXA58*	20 (100 %)	0

Table 4: Detection of the carbapenem resistance genes among the CSAB and CRAB *A. baumannii* isolates

Carbapenemases	**CSAB**	**CRAB**	
	n=7	**n=13**	***p* value**
GES	6 (85.71 %)	10 (76.92 %)	1.00
KPC	4 (57.14 %)	4 (30.77 %)	0.36
NDM	7 (100 %)	10 (76.92 %)	0.52
VIM	6 (85.71 %)	13 (100 %)	0.35
IMP	1 (14.29 %)	2 (15.38 %)	1.00
OXA51	6 (85.71 %)	13 (100 %)	0.35
OXA58	0	0	
SIM	0	0	
GIM	0	0	

3.5. Molecular typing of *A. baumannii* isolates

ERIC-PCR was used to detect the genetic relatedness among the *A. baumannii* isolates. On the agarose gel, all the resulting PCR bands had patterns that ranged from 200 bp to 1500 bp (Fig. 2). Results were analyzed using package MVSP software, and a dendrogram was constructed. The dendrogram depicted in Fig. (3) revealed that the analyzed *A. baumannii* isolates could be classified into 3 distinct clusters; mainly A, B, and C, where the latter includes 2 sub-clusters C1 and C2. The predominant cluster was C which contained 15 isolates (75 %), where C1 included 3 isolates, and C2 included 12 isolates. The remaining 5 isolates have substantially different banding patterns, thus were designated as clusters A

(20 %) and B (5 %). In this study, the XDR isolates were more commonly detected among CRAB with statistical significance ($p \geq 0.01$). Meanwhile, there was no difference in the distribution of ERIC groups between CRAB and CSAB (Table 5). There was no statistically significant difference ($p \geq 0.19$) in the distribution of ERIC groups of the *A. baumannii* isolates according to the type of infection; however, groups A and B were more commonly isolated from patients with diabetic foot than group C. Moreover, *A. baumannii* isolates recovered from patients with abscess; chronic ulcer, diabetic abscess, infected burn, meningitis, and pneumonia were included in ERIC group C only (Table 6). Isolates in Cluster C were consistently more resistant to all the tested antibiotics than those isolates in the other two clusters. Cluster C, was the major cluster that showed higher rate of *KPC*, *NDM* and *IMP* (Table 7). Currently, genotyping and detection of resistance genes have classified the 20 isolates of *A. baumannii* into 4 clusters; mainly A, B, C, and D (Fig. 4). Cluster D could be categorized into 2 subtypes. D2 was the most prevalent subtype, comprising 11(55 %) isolates, expressing several genes, including *OXA51*, *GES*, *NDM*, and *VIM*.

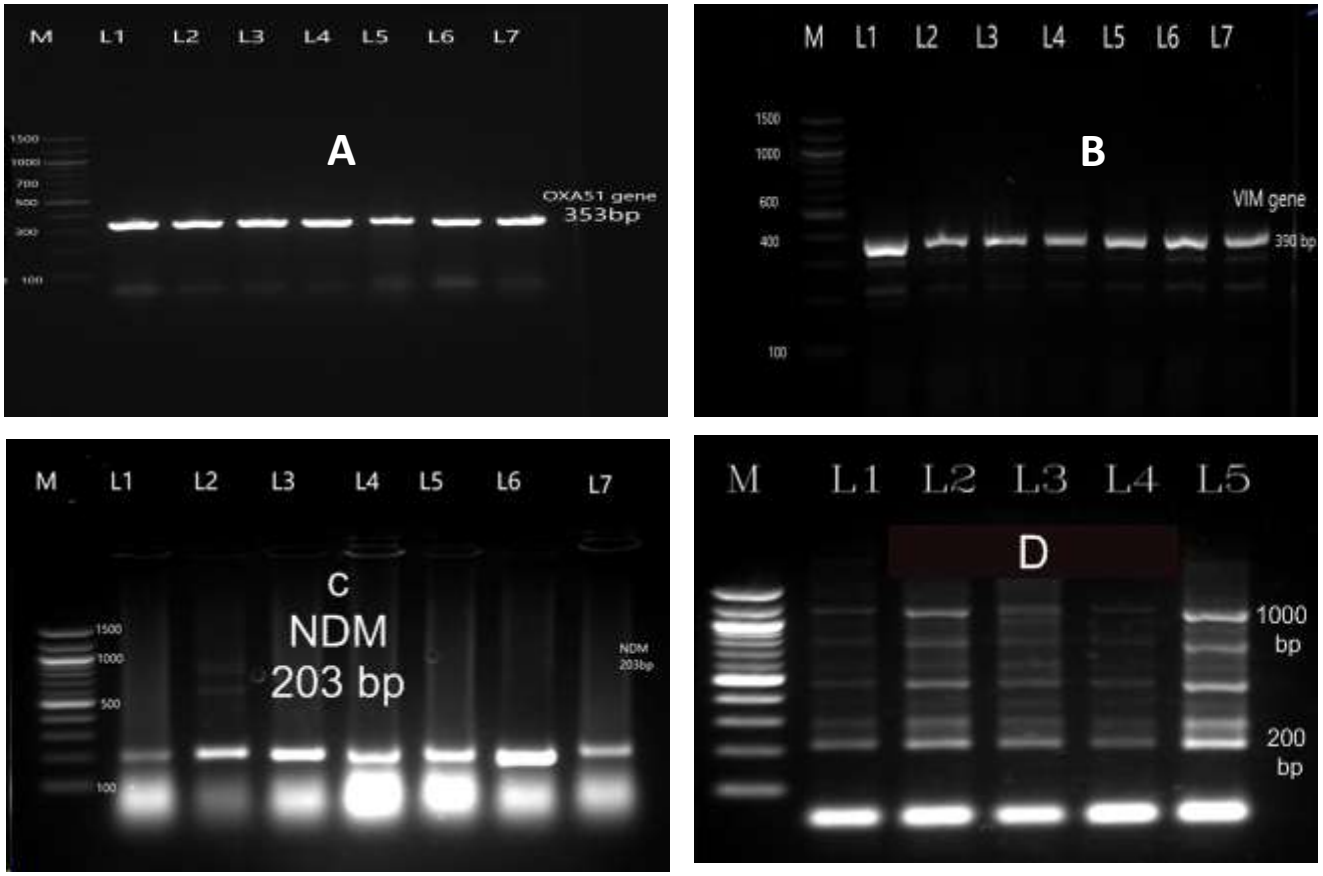

Fig. 2: Agarose gel electrophoresis of the amplified genes using PCR. Where; A: OXA-51 genes (L1-7, 353 bp), B: VIM genes (L1-7, 390 bp), C: NDM genes (L1-7, 203 bp), D: Represent results of ERIC-PCR, to determine the genetic relatedness among the clinical isolates of *A. baumannii*, where (L1-5) show variable PCR banding patterns that range from 200 bp to 1500 bp. M: DNA ladder (100 bp-1500 bp)

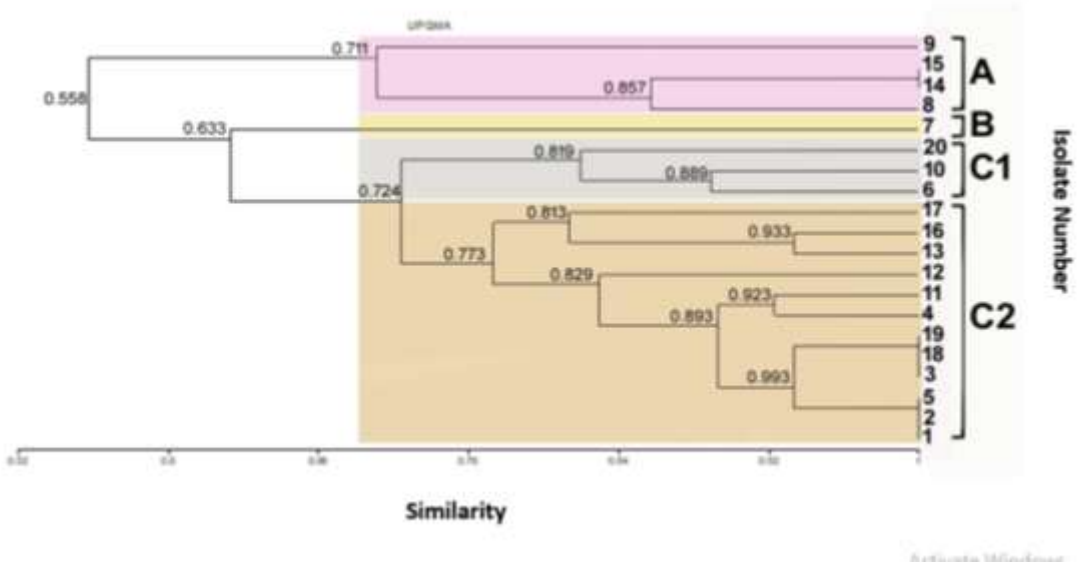

Fig. 3: Dendrogram presenting ERIC-PCR of the *A. baumannii* isolates that revealed the clusters and similarity indexes of the analyzed *A. baumannii* isolates, which were classified into 3 distinct clusters; A (20 %), B (5 %), and C (75 %) that included 2 sub-clusters C1 and C2

Table 5: Relation among the healthcare-associated infections caused by CRAB and CSAB, MDR, XDR, and ERIC groups

Variable	**CSAB** **n=7**	**CRAB** **n=13**	***p* value**
MDR	5 (71.43%)	12 (92.31%)	0.27
XDR	2 (28.57%)	12 (92.31%)	0.01
ERIC groups			
A/ B	2 (28.57 %)	3 (23.00 %)	1.00
C1/ C2	5 (71.43%)	10 (76.92%)	

Table 6: Relation between ERIC groups and the types of infection

Type of infection	**Group (A\ B)** n=5	**Group (C1and C2)** n=15	***p* value**
Respiratory tract infection	2 (40.00 %)	5 (33.33 %)	
Surgical site infection	1 (20.00 %)	3 (20.00 %)	0.19
Diabetic foot	2 (40.00 %)	1 (6.67 %)	
Others	0	6 (40.00 %)	

Table 7: Relation between ERIC groups and the presence of resistance genes

Resistance genes	**Group (A\ B)** n=5	**Group (C1and C2)** n=15	***p* value**
GES	4 (80.00 %)	12 (80.00 %)	1.00
KPC	1 (20.00 %)	7 (46.67 %)	0.60
NDM	4 (80.00 %)	13 (86.67 %)	1.00
VIM	5 (100 %)	14(93.33 %)	1.00
IMP	0	3 (20.00 %)	0.54
OXA-51	5 (100 %)	14 (93.33 %)	1.00
OXA58	0	0	-
SIM	0	0	-
GIM	0	0	-

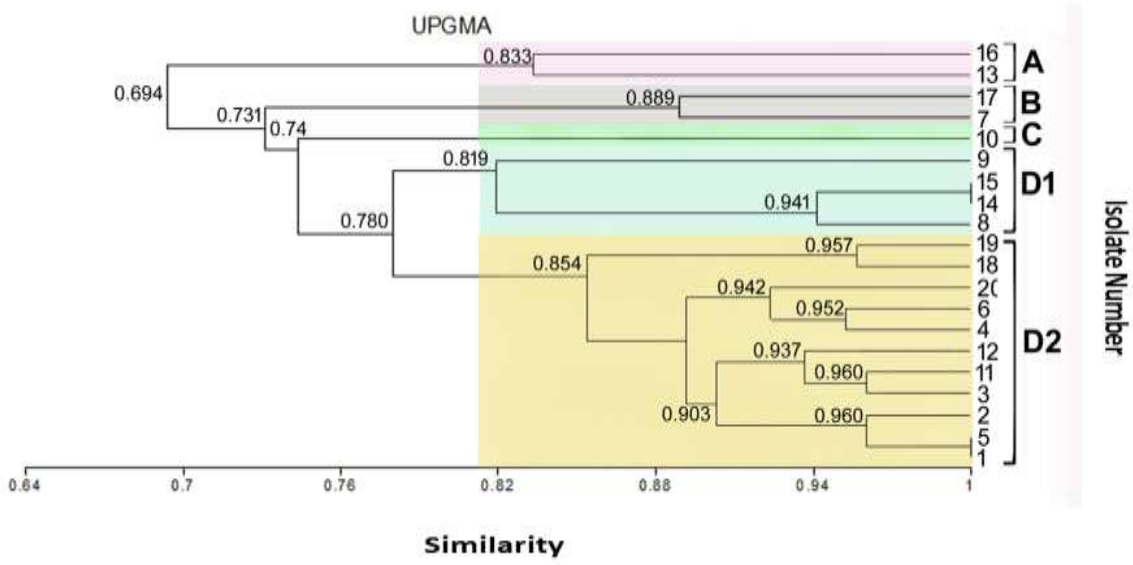

Fig. 4: Dendrogram demonstrating ERIC-PCR plus resistance genes of the *A. baumannii* isolates, which were classified into 4 clusters; mainly A, B, C, and D that was categorized into 2 subtypes D1 and D2, in addition to the isolates similarity indexes

4. Discussion

The resistance rates of *A. baumannii* vary from one country to another; however, they have been increasing over time (Al-Tamimi *et al*., 2022). In the current study, the overall detected resistance to most antibiotics was high, notably to β-lactams; carbapenems, cephalosporins, and fluoroquinolones. The *A. baumannii* isolates retained good susceptibility rate to minocycline and doxycycline antibiotics.

Carbapenems were considered as the last resort for treatment of infections associated with MDR strains, due to their stability against extended spectrum and Ampc β-lactamases (Abd El-Baky *et al*., 2020). In the last few years, carbapenem-resistant bacteria have emerged as a result of the extensive use of these antibiotics (Abd El-Baky *et al*., 2020). In Egypt, the resistance to carbapenems in the clinical *A. baumannii* strains has been noted (Al-Agamy *et al*., 2014). Few previous studies have determined the resistance rates for carbapenems in *A. baumannii* strains in Egypt (Al-Agamy *et al*., 2014).

The present study showed a moderate level of carbapenem resistance (65 %), in comparison with several Egyptian studies, including Al-Hassan *et al*., (2019); Benmahmod *et al*., (2019); Abozahra *et al*., (2021); Eshra, (2021); Ibrahim *et al*., (2022), which reported resistance rates of 95.7 %, 93 %, 98 %, 91.6 %, 88.9 %, and 100 %, respectively.

According to Coskun *et al*., (2019), *A. baumannii* often develops resistance against carbapenems, which play critical roles in the treatment of nosocomial infections caused by the Gram-negative pathogens. The World Health Organization (WHO) and Center for Disease Control and Prevention (CDC) defined CRAB as one of the most urgent health threats (Khuntayaporn *et al*., 2021).

A previous study conducted by Coskun *et al*., (2019) reported that MDR strains are often resistant to carbapenems. Similarly, in this study, the recorded carbapenem resistance in MDR and XDR isolates was 70.58 % and 85.7 %, respectively. Moreover, a recent study of Khuntayaporn *et al*., (2021) reported a higher percentage (> 90 %) of MDR carbapenem resistant isolates, whereas Lean *et al*., (2014) highlighted that 100 % of the MDR isolates were carbapenem resistant.

Currently, in the tested CRAB isolates, molecular analysis of the resistant isolates revealed a markedly elevated prevalence of most of the carbapenemase genes. Genes encoding for bla$_{OXA-51}$ that belong to class D carbapenemases was detected in 100 % of the CRAB isolates, in accordance with several previous studies conducted by Alyamani *et al*., (2015); Hassan *et al*., (2021); Al-Tamimi *et al*., (2022); Massik *et al*., (2022).

There are many reports indicating the presence of bla$_{OXA-58}$ in *A. baumannii* clinical isolates throughout different parts of the world, where different rates of bla$_{OXA-58}$ were previously detected in Tunisia, Egypt, and Algeria, recording 4 %, 9.1 %, and 14.7 %, respectively (Abouelfetouh *et al*., 2019). However, bla$_{OXA-58}$ was absent in all *A. baumannii* isolates collected in this study, in agreement with the previous studies conducted in Mansoura, Egypt, by Benmahmod *et al*., (2019); Abozahra *et al*., (2021). However, other Egyptian studies of Al-Hassan *et al*., (2019); Hassan *et al*., (2021); Wasfi *et al*., (2021) reported different percentages of existence of this gene, recording 3.3 %, 1.9 %, and 2.9 %, respectively. In conclusion, bla$_{OXA-58}$ was absent or existed at low prevalence in several Egyptian studies, suggesting that its role in conferring carbapenem resistance among the tested isolates is limited.

Non-bla$_{OXA}$ carbapenemases can be acquired by *A. baumannii*, including MBLs of Ambler class B; such as the *NDM*-group, and Ambler class A carbapenemases as the *KPC* and *GES* (Ayoub *et al*., 2021). As bla$_{NDM-1}$-carrying bacteria are often considered as MDR; infections due to *NDM-1*-producing *A. baumannii* may increase the risk of poor clinical outcomes (Moyo *et al*., 2021). The presence of bla$_{NDM-1}$ demonstrates a pattern of resistance to all β-lactam agents except for the mono-lactams (Khuntayaporn *et al*., 2021). In this study, bla$_{NDM}$ was detected in 76.92 % of the tested CRAB isolates.

Abouelfetouh *et al*., (2019) have reported that MBLs are especially problematic as their genes are harbored on genetic mobile elements, thus they become easily disseminated among the clinical isolates. Therefore, detection of carbapenemases among the resistant strains is vital to direct the proper treatment regimen.

Concerning Class B carbapenemases, including *IMP* and *VIM*, they were currently detected in 15.38 % and 10 % of the tested CRAB isolates, respectively. Lower rates of both genes were detected by Shirmohammadlou *et al*., (2018), while Eshra, (2021) recently detected lower *VIM* rate and higher *IMP* rate. Regarding the bla$_{GIM}$ and bla$_{SIM}$ genes, none of the tested *A. baumannii* isolates in this study harbored any of these genes. Similar results were also obtained by Vahhabi *et al*., (2021), while several previous studies conducted by Alkasaby and El Sayed, (2017); Wasfi *et al*., (2021) reported higher rates of both genes.

In Egypt, many authors have reported different prevalence rates of MBLs in *A. baumannii* (Hassan *et al*., 2021). Meanwhile, Alkasaby and El Sayed, (2017) highlighted that among 280 clinical *A. baumannii* isolates collected from Egyptian patients admitted to Mansoura University Hospital ICU, 95 % of these isolates harbored MBLs, where bla$_{IMP}$ accounted for 95.7 % of the isolates. A second Egyptian study carried out by Ramadan *et al*., (2018) detected bla$_{NDM}$ among 66.7% of 50 *A. baumannii* isolates; however, neither bla$_{IPM}$ nor bla$_{VIM}$ genes were recorded among the tested isolates. Among about 74 carbapenem resistant *A. baumannii* (CR-AB) isolates collected from different clinical specimens at Alexandria University Hospital in

Egypt, both of bla_{VIM} and bla_{NDM} genes were detected in 100 % and 12.1 % of the isolates, respectively (Abouelfetouh *et al*., 2019).

In the current study, class A carbapenemases such as *KPC* and *GES* were detected in 30.77 % and 76.92 % of the tested CRAB isolates, respectively. These results are in disagreement with the previous studies of Coskun *et al*., (2019); Grisold *et al*., (2021), which did detect neither the *KP*C nor *GE*S in their isolates.

Regarding bla_{GIM} and bla_{SIM} genes, none of the *A. baumannii* isolates in the present study harbored any of these genes, in accordance with the previous studies conducted by Abouelfetouh *et al*., (2019); Hassan *et al*., (2021); Vahhabi *et al*., (2021). The spreading capacity of the bla_{KPC} and bla_{GES} genes is high, due to their linkage to mobile elements such as Tn4401, which is located on the conjugative plasmid and integrin. According to Wasfi *et al*., (2021), infection with bla_{KPC} positive *A. baumannii* is usually linked to a high level of morbidity and mortality.

Currently, the *KPC*, *GES*, *NDM*, *IMP*, *VIM*, and *OXA51* genes were detected in 47.06 %, 82.35 %, 88.24 %, 17.65 %, 94.12 %, and 94.12 % of *A. baumannii* MDR isolates, respectively. A previous study of El-Kazzaz *et al*., (2020) recorded the blaOXA-58 gene in 17.4 % of the MDR strains. In addition, the examined strains harbored *NDM*, *VIM*, and *IMP* genes by 13 %, 43.5 %, and 60.9 %; respectively, however the *GES* and *KPC* genes were not detected. In this study, about 100 % of the MDR isolates carried three or more carbapenemase genes, in consistence with the previous study of Hoang Quoc *et al*., (2019), which revealed that all of the MDR isolates harbored two or more genes.

Statistical analysis of results revealed that there is no correlation between the prevalence of carbapenemase genes and carbapenem resistance, in agreement with Al Amri *et al*., (2020), who obtained the same results. Moreover, it is also observed that there is no relation between presence of the carbapenemase genes and of being MDR, in agreement with a previous study performed on MDR *A. baumannii*, which was conducted by Quoc *et al*., (2019).

In the present study, all the XDR isolates carried both $bla_{OXA\text{-}51}$ and bla_{VIM} genes, while bla_{NDM} and bla_{GES} were detected in 85.71 % and 78.57 % of these isolates, respectively. Statistical analysis of results revealed that there is a statistical correlation that existed between being XDR and being CRAB (p =0.01), which is a unique finding of the study.

ERIC-PCR is a simple and rapid method that has been conducted for distinction among the clinical *A. baumannii* strains (Shali *et al*., 2022), with an acceptable differentiation and repeatability (Zarifi *et al*., 2020). In this study, the dendrogram cluster analysis for the 20 *A. baumannii* isolates distinguished 3 distinct clusters and three single patterns as a type strain. Notably, 75 % of the *A. baumannii* isolates were classified into Cluster C, which is the principal cluster. Regarding resistance of the phenotype, isolates in Cluster C were consistently more resistant to all the tested antibiotics those isolates in the other two clusters. This suggested that a single dominant clone of MDR *A. baumannii* had prevailed in Sohag University Hospital in 2020. Cluster C, showed higher rate of *KPC*, *NDM*, and *IMP* encoding genes. Contamination and cross-transmission within the hospital ward environment may have induced spread of the resistant *A. baumannii*, which necessitates call for greater awareness of implementation and monitoring of infection control measures.

Several Egyptian researches have studied the genetic relatedness among the clinical *A. baumannii* strains using ERIC-PCR. A previous study of Mohammed *et al*., (2020) revealed that the obtained isolates, including 2 Pan drug-resistant (PDR) and 32 selected XDR were divided into three major clusters, with similarities ranging from 0 % to 97.3 %. Cluster I was a major cluster representing 58.82 % of the isolates, and Cluster II depicted 5.88 %. However, Cluster III presented 35.29 % of the total

isolates, which means that these isolates were not clonal.

A second study conducted by Tawfick *et al*., (2020) reported that based on ERIC genotyping of 79 MDR *A. baumannii* isolates, there was no evidence of *A. baumannii* clonal dissemination among the isolates, which indicates that there was a significant molecular heterogeneity between these isolates. In Egypt, Wasfi *et al*., (2021) study revealed that about 34 CRAB isolates were divided into nine ERIC clusters, and this observed diversity of ERIC patterns suggest the dissemination of carbapenem-hydrolyzing genes among the genetically unrelated *A. baumannii* isolates. This dissemination may be attributed to a horizontal gene transfer of plasmids carrying resistance determinants.

Conclusion

The alarmingly higher incidence of MDR and XDR *A. baumannii* isolates that produce carbapenemases calls for stringent infection control strategies. For infection management; local outbreak surveillance, ongoing successful treatment, and monitoring of resistance genes; particularly those of transferrable carbapenemases, are essential. Currently, *A. baumannii* poses a serious health threat in the Egyptian hospitals. In the present work, our findings suggest a critical healthcare problem that may lead to decreasing the efficiency of future therapeutic options. Call for the development of new infection control measures to prevent the spread of these carbapenemases genes among the MDR bacteria, represents an urgent need for new drug combinations and treatment options.

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Conflict of interest

All authors declare non-existence of conflict of interests.

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Ethical approval

Informed written consents were taken from all the patients' and\ or their relatives. Ethical approval was obtained from the Ethical and Scientific committee (Code number: HV21/2020), Faculty of Pharmacy, Minia University, Minia, Egypt.

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