

## ORIGINAL ARTICLE

# Phenotypic and Genotypic Characterization of AmpC Beta Lactamases in *Acinetobacter* Species Isolated from Cairo University Hospitals

<sup>1</sup>Dina M.A. Elsayed\*, <sup>2</sup>Eman A. El-Seidi, <sup>3</sup>Laila A. Rashed, <sup>4</sup>Maha M. Kotb

<sup>1</sup>Department of Medical Microbiology and Immunology, Faculty of Medicine, Helwan University, Ain Helwan, Cairo 11795, Egypt

<sup>2</sup>Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo 11562, Egypt; Basic Medical Sciences Department, Faculty of Medicine, Galala University, Suez, Egypt

<sup>3</sup>Department of Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

<sup>4</sup>Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo 11562, Egypt

## ABSTRACT

### Key words:

*AmpC*  $\beta$ -Lactamase; *AmpC* disk test; Disk potentiation test; Multiplex PCR

### \*Corresponding Author:

Department of Medical Microbiology and Immunology, Faculty of Medicine, Helwan University, Cairo 11795, Egypt.  
Tel.: +201060861434  
[dina.azim@med.helwan.edu.eg](mailto:dina.azim@med.helwan.edu.eg)  
ORCID No.:  
<https://orcid.org/0009-0006-6026-7219>

**Background:** In Egypt, the prevalence of plasmid-mediated class C (*AmpC*) beta-lactamases ( $\beta$ -lactamases) in *Acinetobacter* species is on the rise, and its resistance to a wide range of  $\beta$ -lactam drugs makes it a hazardous condition. **Objectives:** The aim of this research was to assess the occurrence of *AmpC* among *Acinetobacter* clinical isolates by phenotypic confirmatory tests and to compare them with PCR as the gold standard method. **Methodology:** The current study incorporated 50 *Acinetobacter* clinical isolates. Detection of *AmpC*-producing *Acinetobacter* isolates was done by two phenotypic methods; *AmpC* disk test with Tris-EDTA and disk potentiation test (DPT) with aminophenyl boronic acid (APB) and then compared with genotypic detection of plasmid-mediated *AmpC* (*pAmpC*) gene(s) families by multiplex PCR as the gold reference technique. **Results:** According to the study's findings, the frequency of *AmpC*-producing isolates was 68.8% by *AmpC* disk test, 72.9% by DPT with APB test, and 79.2% by PCR. The sensitivity of *AmpC* disk test and DPT with APB test was 86.8% and 92.1% respectively and the specificity was 100% for both tests. The CIT family of *AmpC* genes was the most common type to be found in the study (33.3%). **Conclusion:** Phenotypic detection of *AmpC* was more sensitive by DPT with APB test than the *AmpC* disk test with Tris-EDTA. Phenotypic methods are influenced by false-negative results. Therefore, PCR remains the gold standard for detecting different forms of *pAmpC* gene families and determining their precise prevalence, enabling the taking of suitable actions to restrict the spread of resistant microorganisms.

## INTRODUCTION

*Acinetobacter baumannii* takes the lead as the predominant species (spp.) incorporated in outbreaks and hospital-acquired infections (HAIs), specifically in intensive care units (ICUs), notably in cases of ventilator-associated pneumonia (VAP). The rise of multidrug-resistant (MDR) *Acinetobacter* spp. has been aided by inappropriate usage of different antibiotics<sup>1</sup>. Unfortunately, different classes of plasmid- and chromosomally-mediated beta-lactamases ( $\beta$ -lactamases) have been discovered in *A. baumannii*, reducing the effectiveness of this class of antibiotics in the fight against their infection<sup>2</sup>. Among the main resistance determinants found in *A. baumannii* are class C  $\beta$ -lactamases (*AmpC*)<sup>3</sup>. The ability of *AmpC*  $\beta$ -lactamases to deactivate cephamycins and other extended-spectrum cephalosporins (ESCs) as well as

their resistance to clavulanic acid distinguish them from other  $\beta$ -lactamases<sup>4</sup>.

Infections caused by *Acinetobacter* spp. that produce plasmid-mediated *AmpC* (*pAmpC*)  $\beta$ -lactamases generally exhibit resistance against all  $\beta$ -lactams; however, cefepime, ceftazidime, and carbapenems offer few therapeutic options<sup>5</sup>. The Clinical and Laboratory Standards Institute (CLSI) has not issued any guidelines concerning the use of phenotypic methods in the investigation of organisms that produce *AmpC*<sup>6</sup>. Several *AmpC*  $\beta$ -lactamase detection methods have been proposed; resistance to ceftazidime and/or cephamycins<sup>7</sup>, preserving susceptibility to cefepime<sup>8</sup>, amended cefoxitin Hodge assay<sup>9</sup>, *AmpC* disk test with Tris-EDTA<sup>10</sup>, inhibitor-based tests [like boronic acid (BA) compounds]<sup>11</sup> or cloxacillin<sup>12</sup>, and rapid chromogenic assays<sup>13</sup>.

Unfortunately, these methods prove inadequate for routine clinical application in microbiology laboratories

and fail to detect all types of AmpC  $\beta$ -lactamases<sup>14</sup>. *Acinetobacter* AmpC enzymes are detected by cefoxitin (FOX) screening and then by the confirmatory phenotypic tests<sup>15,16</sup>. However, chromosomal *ampC* genes and *pampC* genes cannot be distinguished using phenotypic assays<sup>10,17</sup>. The presence of *ampC* genes can be detected *via* polymerase chain reaction (PCR) analysis. Six pAmpC gene families (ACC, DHA, CIT, EBC, FOX, and MOX) were identified by using PCR<sup>18</sup>.

## METHODOLOGY

The current study was performed between November 2019 and April 2021. A total of 50 *Acinetobacter* clinical isolates, acquired from the Strain Bank, Medical Microbiology & Immunology Department, Faculty of Medicine, Cairo University were used. These isolates were recovered from various clinical samples from hospitalized patients at Kasr Al Ainy University Hospitals. The study received approval from the Research Ethics Committee of the Institutional Review Board, Faculty of Medicine, Cairo University. Further identification of all *Acinetobacter* isolates up to spp. level and antibiotic susceptibility testing (AST) was done at the Department of Microbiology of Theodor Bilharz Research Institute (TBRI) by the automated VITEK® 2 compact system (bioMérieux, Marcy l'Etoile, France)<sup>19</sup>.

The phenotypic tests were done at the Medical Microbiology & Immunology, Faculty of Medicine, Cairo University. Screening for AmpC production was performed by using the modified Kirby Bauer (KB) disk diffusion method using FOX disks (30 $\mu$ g) (Oxoid, UK). According to CLSI criteria, isolates demonstrating resistance or intermediate resistance to FOX (zone diameter  $\leq$  18 mm) were deemed to be potential AmpC producers<sup>6,16</sup>. All of the isolates that tested positive on the screening method underwent two further confirmatory phenotypic tests.: AmpC disk test with Tris-EDTA and DPT with APB test<sup>16</sup>.

### AmpC disk test with Tris-EDTA:

The test was done following the Black et al.<sup>10</sup> method by using FOX-sensitive *E. coli* ATCC 25922 (obtained from the Microbiology Department of TBRI, Egypt). On a Mueller Hinton Agar (MHA) plate, *E. coli* ATCC 25922 was cultivated as a lawn culture (Becton-Dickinson, USA) plate. Many colonies of the test organism were inoculated onto 6 mm sterile disks moistened with 20  $\mu$ l of a 1:1 saline and 100 X Tris-EDTA solution (Sigma-Aldrich, St. Louis, USA). On the agar plate, the inoculated disk was put next to a FOX disk, and incubation was performed at 35°C.

Positive results were determined when there was an indentation or flattening in the inhibitory zones on the plates, indicating enzymatic inactivation of FOX, and negative results were determined when there was no visible indentation, indicating no enzymatic inactivation of FOX.

### Disk potentiation test with Aminophenylboronic acid:

The MHA plate was swabbed with a colony of the test isolate. A ceftazidime (CAZ) disk (Oxoid, UK) was infused with 300 $\mu$ g of APB after it had been previously dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA) at an ultimate concentration of 100mg/ml and a final volume of 0.3ml per disk. These disks (CAZ+APB) were put on MHA plates with CAZ disks at a 30 mm distance from center to center. The plates were then put in an incubator at 37°C overnight. The presence of a 5 mm or increased inhibitory zone around the disk containing both CAZ and APB compared to that containing CAZ alone was considered positive (i.e., AmpC producer)<sup>14</sup>.

### Genotypic detection of *Acinetobacter* isolates harboring family-specific plasmid-mediated *ampC* gene(s) by multiplex PCR:

The genotypic method was done at the Medical Biochemistry Department (Molecular Biology Unit), Faculty of Medicine, Cairo University. Isolates that were screened positive by FOX disks were freshly subcultured on blood agar plates before performing DNA extraction. Bacterial DNA extraction was performed using the DNASure® Tissue Mini Kit (Genetix, New Delhi) following the instructions provided by the manufacturer.

Multiplex PCR technique was achieved to detect *Acinetobacter* isolates harboring family-specific *pampC* gene(s) according to Pérez-Pérez and Hanson<sup>18</sup>. The final volume for the PCR reaction was 50  $\mu$ l. The primers (6 pairs) used for PCR amplification; were manufactured by (Vivantis, Malaysia) and are listed in (Table 1). Each reaction tube contained 25  $\mu$ l 2X Taq master mix: [Taq DNA polymerase (0.05u/ $\mu$ l), 2X ViBuffer A (100mM KCl, 20mM TrisHCl (pH9.1 at 20°C) and (0.02% Triton™ X-100), 0.4 mM dNTPs, 3 mM MgCl<sub>2</sub>] (Vivantis, Malaysia), 17  $\mu$ l nuclease-free water, 0.25  $\mu$ l of each primer, and 5  $\mu$ l template DNA.

Using the thermal cycler (Biometra, Göttingen, Germany), the PCR cycling conditions involved a preliminary denaturation step (for 5 mins at 94°C). This is followed by 25 cycles of DNA denaturation for 30s at 94°C, primer annealing for 30s at 61°C, and primer extension for 30s at 72°C. Subsequently, a final extension step for 7 mins at 72°C was done<sup>18</sup>.

**Table 1: Primers used for the detection of family-specific *pampC* genes in *Acinetobacter* by multiplex PCR<sup>18</sup>.**

Primer	Sequence (5' to 3')	Target (s)	Expected amplicon size (bp)
MOXMF	GCT GCT CAA GGA GCA CAG GAT	MOX-1, MOX-2, CMY-1 CMY-8 to CMY-11	520
MOXMR	CAC ATT GAC ATA GGT GTG GTG C	MOX-1, MOX-2, CMY-1 CMY-8 To CMY-11	
CITMF	TGG CCA GAA CTG ACA GGC AAA	LAT-1 to LAT-4 CMY-2 to CMY-7-BIL-1	462
CITMR	TTT CTC CTG AAC GTG GCT GGC	LAT-1 to LAT-4 CMY-2 to CMY-7-BIL-1	
DHAMF	AAC TTT CAC AGG TGT GCT GGG T	DHA-1, DHA-2	405
DHAMR	CCG TAC GCA TAC TGG CTT TGC	DHA-1, DHA-2	
ACCMF	AAC AGC CTC AGC AGC CGG TTA	ACC	346
ACCMR	TTC GCC GCA ATC ATC CCT AGC	ACC	
EBCMF	TCG GTA AAG CCG ATG TTG CGG	MIR-1T, ACT-1	302
EBCMR	CTT CCA CTG CGG CTG CCA GTT	MIR-1T, ACT-1	
FOXMF	AAC ATG GGG TAT CAG GGA GAT G	FOX-1 to FOX-5b	190
FOXMR	CAA AGC GCG TAA CCG GAT TGG	FOX-1 to FOX-5b	

Agarose gel electrophoresis was used to separate the amplified PCR products (**Scie-Plas, Cambridge, UK**) and then visualized under UV illumination, and photographed with the Bio-Rad ChemiDoc Gel Documentation system (**Bio-Rad, California, USA**) as previously described by Liu and Liu<sup>20</sup>.

#### Statistical analysis

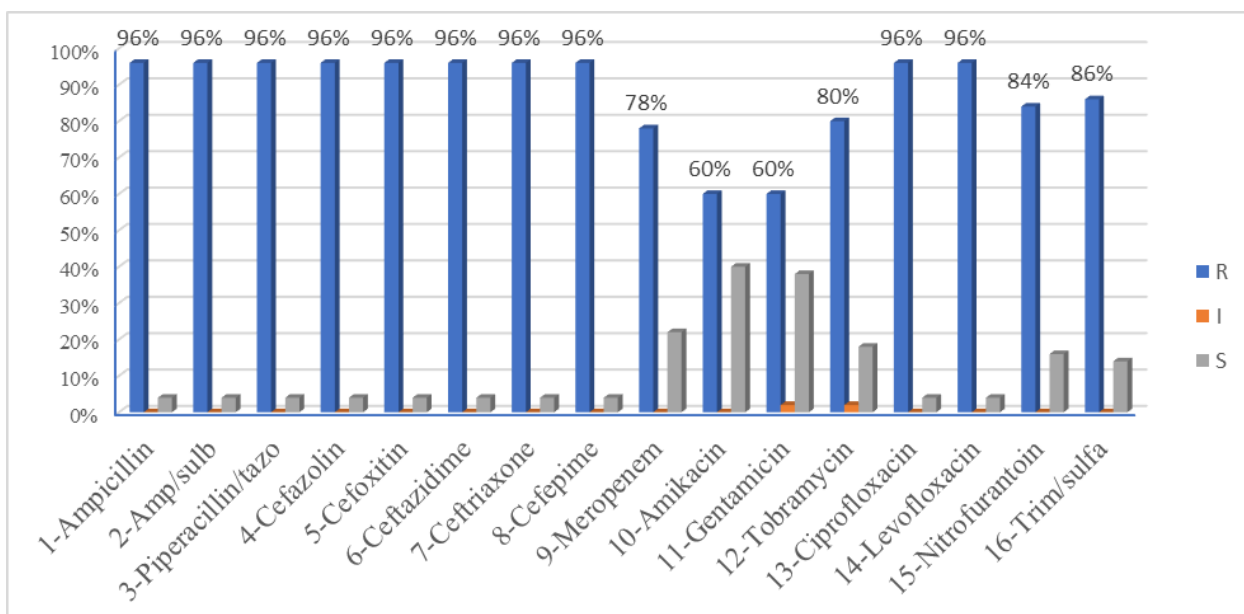
Statistical Package for the Social Sciences (SPSS) version 26 was used for data coding and entry (**IBM Corp., Armonk, NY, USA**). Data summarizing was accomplished by employing absolute and relative frequencies (counts and percentages). Standard diagnostic indices like specificity, sensitivity, negative predictive value (NPV), positive predictive value (PPV), and diagnostic efficacy were computed. To assess the agreement between categorical variables, the Kappa measure of agreement was utilized. Statistical significance was determined by considering a p-value below 0.05<sup>21</sup>.

## RESULTS

Isolates of *Acinetobacter* were obtained from the following specimens: 16 (32%) sputum, 11 (22%) urine, 11 (22%) blood, 9 (18%) endotracheal aspirate, and 3 (6%) from wound swabs. The following isolates were isolated from patients with distinct types of infections:

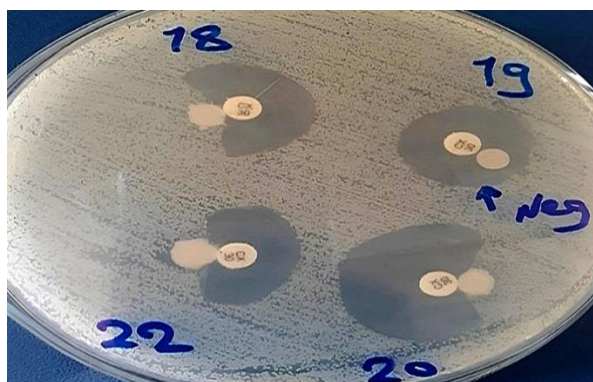
15 (30%) had respiratory tract infections (RTI), 11 (22%) had septicemia, 10 (20%) had VAP, 6 (12%) had catheter-associated urinary tract infections (CAUTI), 5 (10%) had non-catheter associated UTI and 3 (6%) had skin and soft tissues infection.

Identification of all 50 *Acinetobacter* isolates revealed that 42 isolates (84%) were *A. baumannii*, while 8 isolates (16%) were identified as *A. baumannii* complex. Due to the difficulty in phenotypic differentiation between spp., the term *A. baumannii* complex has been used and it is made up of five spp. that are phenotypically and genotypically related, with valid names: *A. baumannii*, *A. nosocomialis*, *A. pittii*, *A. calcoaceticus*, *A. seifertii*<sup>22</sup>. Antibiotic susceptibility testing revealed widespread resistance to antibiotics, whereas 48 isolates (96%) were resistant to cefoxitin (FOX), piperacillin/tazobactam (TZP), ampicillin/sulbactam (SAM), ampicillin (AMP), cefazolin (CZ), ceftriaxone (CRO), ceftazidime (CAZ), and cefepime (FEP). Meropenem (MEM) showed the least resistance rate; 39 (78%) of all tested  $\beta$ -lactam antibiotics. The resistance among non- $\beta$ -lactam antibiotics was 48 (96%) to ciprofloxacin (CIP) and levofloxacin (LEV), 43 (86%) to trimethoprim/sulfamethoxazole (SXT), 42 (84%) to nitrofurantoin (NFN), 40 (80%) to tobramycin (TOB), and 30 (60%) to each of amikacin (AK) and gentamicin (CN) (**Figure 1**).

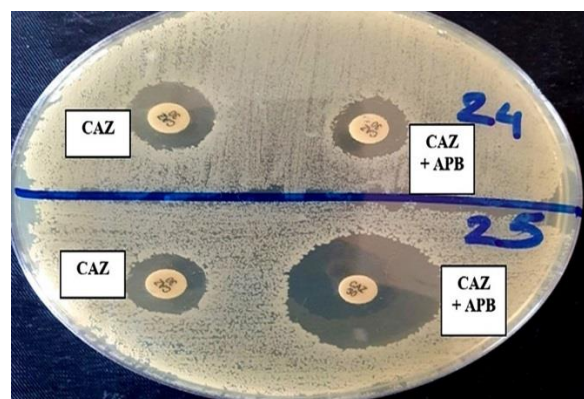


**Fig. 1:** VITEK® 2 compact system AST results.

Screening of all the 50 *Acinetobacter* isolates revealed that 48 isolates (96%) were resistant to FOX by showing the diameter of the inhibitory zone surrounding the disk is  $\leq 18$  mm (potential AmpC producers) and out of all the 48 screened positive isolates by FOX disks, AmpC phenotype was confirmed in 33 (68.8%) and 35 (72.9%) of the isolates using the AmpC disk test with Tris-EDTA and DPT with APB test respectively (Figure 2) (Figure 3).

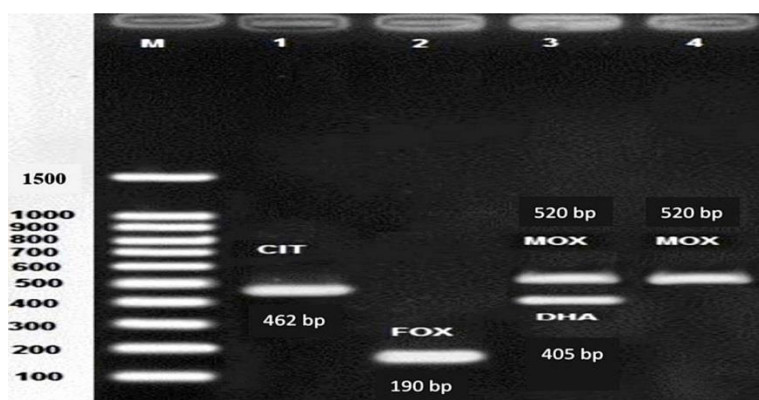


**Fig. 2:** AmpC disk test. AmpC-producing *Acinetobacter* strains (18, 20, and 22) demonstrated positive AmpC disk test by either indentation or flattening in the ceftaxime (CX) inhibitory zones, while negative AmpC test (isolate 19) with neither indentation nor flattening in the ceftaxime (CX) inhibitory zones.



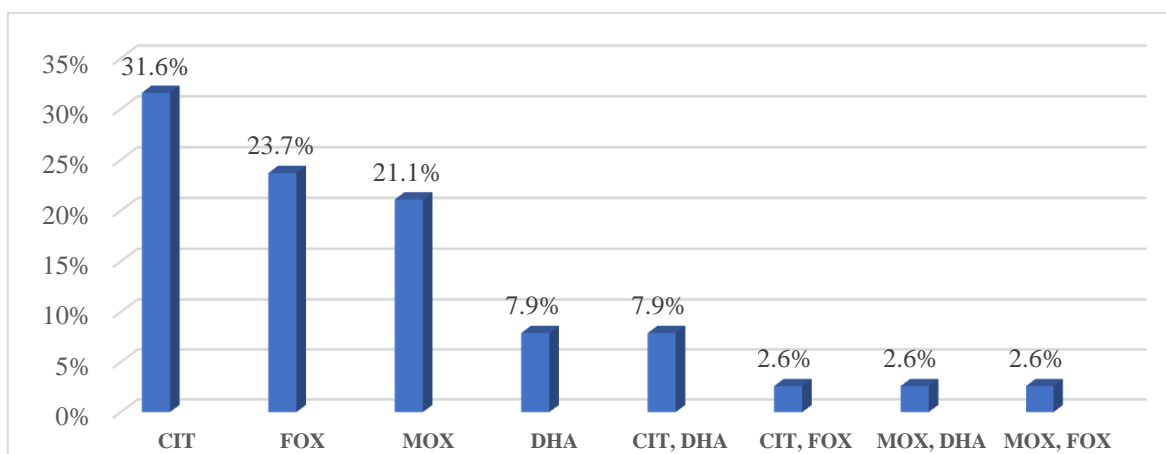
**Figure 3:** Disk potentiation test (DPT) with Aminophenylboronic acid (APB). Negative test (top) and positive test (bottom). In the positive test, the zone of inhibition around the disk containing both CAZ and APB is greater than that containing CAZ alone by  $\geq 5$  mm, demonstrating AmpC-producing *Acinetobacter* strains.

Out of the 48 screened positive isolates by FOX disks, 38 (79%) were positive for the presence of *pampC* gene(s) by PCR. The most predominant *pampC* gene was that of the CIT family and was identified in 16 of the isolates (33.3%), followed by the FOX family; detected in 11 isolates (22.9%), then MOX 10 (20.8%), then DHA family 7 (14.6%). There were 6 isolates detected harboring more than one gene. No genes associated with either ACC or EBC families were identified (Figure 4) (Figure 5).



**Figure 4:** Gel electrophoresis of PCR detection of *pampC* genes.

Lane 1: positive isolate harboring *cit* gene as denoted by a positive band at 462 bp  
 Lane 2: positive isolate harboring *fox* gene as denoted by a positive band at 190 bp  
 Lane 3: positive isolate harboring both *mox* and *dha* gene as denoted by a positive band at 520 and 405 bp  
 Lane 4: positive isolate harboring *mox* gene as denoted by a positive band at 520 bp



**Fig. 5:** Distribution of *pampC* genes within the positive isolates by PCR.

Both phenotypic testing techniques revealed a statistically significant (*p*-value 0.001) difference between the two outcomes. The AmpC disk test

displayed a sensitivity of 86.8%, while DPT with APB test was more sensitive and displayed a sensitivity of 92.1%. Both tests showed 100% specificity (**Table 2**).

**Table 2: Correlation between results of confirmatory phenotypic tests and PCR as the gold standard**

		PCR		Kappa	P-value
		Positive	Negative		
AmpC disk test	Positive	33 (86.8%)	0 (0%)	0.733*	<0.001***
	Negative	5 (13.2%)	10 (100%)		
DPT with APB	Positive	35 (92.1%)	0 (0%)	0.829**	<0.001
	Negative	3 (7.9%)	10 (100%)		
Total (48 isolates)		38	10		

\* Kappa 0.733 is considered a good agreement.

\*\*Kappa 0.829 is considered a very good agreement.

\*\*\*P-value < 0.05 is considered statistically significant.

## DISCUSSION

In the current study, AST revealed an MDR pattern of *A. baumannii*. The MDR pattern of AmpC *A. baumannii* revealed by the AST could be related to the fact that the 50 strains of *A. baumannii* were mostly isolated from the ICU. In this study, the resistance of *A. baumannii* isolates to meropenem (MEM) was 39 isolates (78%), which could be linked to the spread of carbapenems' resistance of the OXA type in *Acinetobacter*<sup>20</sup>. The current study's findings were in agreement with Khalifa et al.<sup>23</sup> who reported that 72.3% of the studied isolates were MEM resistant. The study done by Ibrahim et al.<sup>24</sup> also demonstrated a 93.3% resistance to MEM.

In the present study, out of the 48 FOX-resistant isolates, 33 (68.8%) tested positive for AmpC by the AmpC disk test with Tris-EDTA and 35 isolates (72.9%) were AmpC positive by DPT with APB test. In line with the findings of the present study, *Sofy et al.*,<sup>16</sup> found that 67.18% of FOX-resistant *A. baumannii* isolates were AmpC producers by AmpC disk test, while 78.1% of the isolates were AmpC producers by DPT with APB test. In contrast, much lower prevalence rates were documented by Batra et al.<sup>25</sup> who reported that 12.9% of FOX-resistant *A. baumannii* isolates were positive by AmpC disk test, while 12.3% were positive by BA inhibition test. A much higher prevalence rate was found by Eshra et al.<sup>26</sup> who detected 85.4% positivity by AmpC disk test.

Many studies considered PCR as the gold standard method for evaluating other procedures and approaches for identifying AmpC  $\beta$ -lactamases<sup>25,27</sup>. In the current study, multiplex PCR demonstrated that 38 isolates (79.2%) were found positive for the presence of *pampC* genes. On comparing both phenotypic methods used in the current study with PCR; DPT with APB test showed higher sensitivity (92.1%) than AmpC disk test (86.8%), however, the specificity was 100% for both methods. The study by Wookeun et al.<sup>28</sup> indicated similar findings when comparing the AmpC disk test with the BA inhibition test. They found that better results were obtained by using the BA inhibition test (sensitivity 98.4%, specificity 92.2%) than the AmpC disk test (sensitivity 80.3%, specificity 91.6%).

It is worth highlighting that phenotypic methods may yield false-negative results due to the potential scenario where the genes are detectable through PCR, but their phenotypic expression remains undetectable<sup>5</sup>. Testing with APB was very straightforward, extremely sensitive, and incredibly focused; as a result, it is completely appropriate for routine usage in clinical microbiology laboratories<sup>14</sup>. However, Helmy and Wasfi<sup>5</sup> noticed that the AmpC disk test with Tris-EDTA showed higher specificity (100%) and sensitivity (78.3%) than the BA inhibition test which showed

65.2% sensitivity and 73.9% specificity on the studied FOX-resistant isolates.

In the current study, family-specific *pampC*  $\beta$ -lactamase gene(s) were detected in 38 *Acinetobacter* isolates (76%) by multiplex PCR. This agreed with Barwa et al.<sup>29</sup> who reported a 60% prevalence of *pampC* genes in their study. Lower prevalence rates of *pampC* genes were reported by Helmy and Wasfi<sup>5</sup>, and Ghonaim and Moaety<sup>17</sup> who reported 27%, and 28.5%, respectively. The reasons that not all FOX-resistant isolates were detected to be harboring *pampC*  $\beta$ -lactamase gene(s) by PCR can be explained by the following; numerous enzymatic and non-enzymatic processes, including porin channel mutation, metallo  $\beta$ -lactamases (MBLs), and extended-spectrum  $\beta$ -lactamases (ESBLs) can result in FOX resistance<sup>24,30</sup>.

In the present study, CIT is the most predominant gene family detected by multiplex PCR and was detected in 16 isolates (33.3%) followed by FOX which was detected in 11 isolates (22.9%), MOX detected in 10 isolates (20.8%), and DHA detected in 7 isolates (14.6%). In Egypt, these findings were in line with research conducted by Fam et al.,<sup>31</sup> and Helmy and Wasfi<sup>5</sup>, whereby CIT was found in 76.5%, and 86.9% of the studied isolates, respectively. In the current study, no genes from the ACC or EBC families were found, and this was consistent with research by *Gupta et al.*,<sup>32</sup> and *Batra et al.*,<sup>25</sup> which found no evidence of the ACC or EBC family genes. The EBC-type AmpC  $\beta$ -lactamase was found to be more prevalent in *Klebsiella* spp<sup>32</sup>.

## CONCLUSION & RECOMMENDATIONS

Phenotypic detection of AmpC was more sensitive by DPT with APB test than the AmpC disk test with Tris-EDTA. False-negative results in phenotypic methods can occur due to the genes being detectable by PCR but not exhibiting phenotypic expression. Therefore, PCR remains the gold standard for detecting different forms of pAmpC gene families and determining their precise prevalence, enabling the taking of suitable actions to restrict the spread of resistant microorganisms. More surveillance studies on a larger scale on *pampC* genes and the quick development of innovative therapeutic agents to combat MDR pathogens are critical.

### Conflict of Interest:

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

manuscript, and they have approved the manuscript as submitted.

#### Author Contributions:

Dina Mohamed Abdelazim Elsayed: Carried out the experiments, performed the analysis, discussed the results, and wrote the final manuscript with input from all authors.

Maha Mahmoud Kotb: Conceived the original idea, planned and supervised the experiments, discussed the results, revised the work, and approved the final version to be published.

Laila Ahmed Rashed: Supervised the findings of this work, revised the work for important intellectual content, and approved the final version to be published.

Eman Ahmed El-Seidi: Supervised the findings of this work, revised the work critically for important intellectual content, discussed the results, and approved the final version to be published.

## REFERENCES

- Xie M, Chen K, Chan EW-C, Chen S. Synergistic Antimicrobial Effect of Colistin in Combination with Econazole against Multidrug-Resistant *Acinetobacter baumannii* and Its Persisters. *Microbiol Spectr* 2022;10:e0093722–e0093722.
- Kuo H-Y, Chang K-C, Kuo J-W, Yueh H-W, Liou M-L. Imipenem: a potent inducer of multidrug resistance in *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2012;39:33–8.
- Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010;65:233–8.
- Rattan A, Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, et al. Evaluation of Methods for AmpC Beta-Lactamase in Gram Negative Clinical Isolates from Tertiary Care Hospitals. *Indian J Med Microbiol* 2005;23:120.
- Helmy MM, Wasfi R. Phenotypic and molecular characterization of plasmid mediated AmpC  $\beta$ -lactamases among *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* isolated from urinary tract infections in Egyptian hospitals. *Biomed Res Int* 2014;2014:171548.
- CLSI. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 31ST edn. Wayne, America: CLSI supplement M100, 2021.
- Moland ES, Black JA, Ourada J, Reisbig MD, Hanson ND, Thomson KS. Occurrence of newer beta-lactamases in *Klebsiella pneumoniae* isolates from 24 U.S. hospitals. *Antimicrob Agents Chemother* 2002;46:3837–42.
- Doi Y, Paterson DL. Detection of plasmid-mediated class C  $\beta$ -lactamases. *Int J Infect Dis* 2007;11:191–7.
- Yong D, Park R, Yum JH, Lee K, Choi EC, Chong Y. Further modification of the Hodge test to screen AmpC  $\beta$ -lactamase (CMY-1)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*. *J Microbiol Methods* 2002;51:407–10.
- Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC beta-lactamases in Enterobacteriaceae lacking chromosomal AmpC beta-lactamases. *J Clin Microbiol* 2005;43:3110–3.
- Tan TY, Ng LSY, He J, Koh TH, Hsu LY. Evaluation of screening methods to detect plasmid-mediated AmpC in *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. *Antimicrob Agents Chemother* 2009;53:146–9.
- Brenwald NP, Jevons G, Andrews J, Ang L, Fraise AP. Disc methods for detecting AmpC  $\beta$ -lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2005;56:600–1.
- Livermore DM, Warner M, Mushtaq S. Evaluation of the chromogenic Cica- -Test for detecting extended-spectrum, AmpC and metallo- -lactamases. *J Antimicrob Chemother* 2007;60:1375–9.
- Yagi T, Wachino J, Kurokawa H, Suzuki S, Yamane K, Doi Y, et al. Practical methods using boronic acid compounds for identification of class C beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli*. *J Clin Microbiol* 2005;43:2551–8.
- Kumar E, Usha K, Chaudhury A, Ramana B V, Gopal DVRS. Detection of AmpC  $\beta$ -lactamases production in *Acinetobacter* species by inhibitor (disk) based & modified three dimensional (enzyme extraction) methods. *Indian J Med Res* 2014;140:688.
- Sofy KA, Saafan AE, AbdelGhani SM, Amin MA. Phenotypic and genotypic characterization of different classes of  $\beta$ -lactamases amongst *Acinetobacter* spp. isolated from Egyptian hospitals. *N Egypt J Microbiol* 2015;42:36.
- Ghonaim RA, Moaety HA. Comparison between Multiplex PCR and Phenotypic Detection Methods for Identifying AmpC B-lactamases Among Clinical Isolates of Enterobacteriaceae in Zagazig University Hospitals, Egypt. *Clin Microbiol Open Access* 2018;07.
- Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002;40:2153–62.

19. Nimer NA, Al-Saa'da RJ, Abuelaish O. Accuracy of the VITEK 2 system for a rapid and direct identification and susceptibility testing of gram-negative rods and gram-positive cocci in blood samples. *EMHJ-Eastern Mediterr Heal J* 2016;22:193–200.
20. Liu X-Q, Liu Y-R. Detection and genotype analysis of AmpC  $\beta$ -lactamase in *Klebsiella pneumoniae* from tertiary hospitals. *Exp Ther Med* 2016;12:480–4.
21. Galen RS. Predictive Value and Efficiency of Laboratory Testing. *Pediatr Clin North Am* 1980;27:861–9.
22. Koneman E, Procop G, Church D, Hall G, Janda W, Schreckenberger P, et al. The non fermentative Gram negative bacilli. In: Koneman E, Procop G, Church D, Hall G, Janda W, Schreckenberger P, et al., eds. *Koneman's color atlas and textbook of diagnostic microbiology*, 7th ed. 7th ed. Philadelphia: Wolters Kluwer Health, 2017:385–7.
23. Khalifa RA, Aljahdali H, Alghamdi H, Ismail B, Joharjy H, Eibani K. Characterization of  $\beta$ -Lactamase Genes among Multidrug-and Extensively Drug-Resistant *Acinetobacter* and *Enterobacteriaceae* Species Causing Infections in Critically Ill Patient. *Egypt J Med Microbiol* 2023;32:113–23.
24. Ibrahim MA, Abdallah S, Soliman IS, Afifi N, Atiat Allah MNA. Hospital-Acquired Infection by Carbapenem-Resistant *Acinetobacter* Species in ICUs in Assiut, Egypt. *Egypt J Med Microbiol* 2023;32:97–104.
25. Batra P, Khurana S, Govindaswamy A, Aravinda A, Bajpai V, Ayyanar M, et al. Antibiotic resistance profile and co-production of extended spectrum beta lactamases and AmpC in *Acinetobacter* spp. in a level 1 trauma center from India. *J Lab Physicians* 2019;11:128–32.
26. Eshra KA. Phenotypic Methods and Molecular Metallo- $\beta$ -lactamases Genes Detection in Different Clinical Carbapenems Resistant *Acinetobacter* Isolates from Tanta University Hospitals. *Egypt J Med Microbiol* 2021;30:89–95.
27. Alizadeh S, Baserisalehi M, Bahador N. Antibiotic Resistance Patterns and Distribution of Ampc  $\beta$ -Lactamases Genes Among *Acinetobacter Baumannii* Clinical Isolates from Hospitals of Tehran, Iran. 2021.
28. Lee W, Jung B, Hong SG, Song W, Jeong SH, Lee K, et al. Comparison of 3 phenotypic detection methods for identifying plasmid-mediated AmpC betalactamase-producing *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* strains. *Korean J Lab Med* 2009;29:448–54.
29. Barwa R, Abdelmegeed E, Abd EGK. Occurrence and detection of AmpC -lactamases among some clinical isolates of *Enterobacteriaceae* obtained from Mansoura University Hospitals, Egypt. *African J Microbiol Res* 2012;6:6924–30.
30. Rawat V, Singhai M, Kumar A, Jha PK, Goyal R. Bacteriological and resistance profile in isolates from diabetic patients. *N Am J Med Sci* 2012;4:563–8.
31. Fam N, Gamal D, Said M El, Aboul-Fadl L, Dabei E El, Attar S El, et al. Detection of plasmid-mediated AmpC beta-lactamases in clinically significant bacterial isolates in a research institute hospital in Egypt. *Life Sci J* 2013;10:2294–304.
32. Gupta TB, Shariff M, Thukral SS. Identification of AMPC B-lactamase-producing clinical isolates of *Escherichia coli*. *Asian J Pharm Clin Res* 2017;10:357–61.