Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (9) Issue (1) (2024), (125-132) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg

A Novel Method for Rapid Identification of Acinetobacter baumannii Among ICU Patients

Sara. S. Abdelrahman[2], Adel. M. Agha[1] and Sahar. M. Fayed[1]
[1]Clinical and Chemical Pathology Department, Faculty of Medicine, Benha University, Benha, Egypt
[2]Clinical and Chemical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
E-Mail: sarahsamirkh88@gmail.com

Abstract

Background: Acinetobacter baumannii, a nosocomial pathogen, causes severe ICU infections. It's a non-fermentative, gram-negative bacterium. This study aimed to evaluate PCR's efficiency for rapid A. baumannii diagnosis, detect biofilm formation, and assess antimicrobial susceptibility in ICU-isolated A. baumannii. **Methods:** This cross-sectional study involved 40 isolates of Acinetobacter baumannii bacteria from patients who developed clinical evidence of nosocomial infection. Different types of specimens were collected as follows: Lower respiratory tract specimens (25), Blood samples (10), Urine samples (3) and pus samples from infected surgical and traumatic wounds (2). Diagnosis is done by conventional methods, BD phoenix M50 and PCR for detection of specific sequence of Acinetobacter baumannii (gyrB gene sequences). Biofilm formation was assessed as a virulance factor using 96-well microtiter plates and tube method. **Results:** PCR and BD Phoenix™ M50 both showed high agreement with culture for detecting Acinetobacter, scoring perfect (1) and excellent (0.979) respectively. PCR demonstrated 100% accuracy, NPV, PPV, specificity, and sensitivity, while Phoenix showed 98.4 NPV, 100% accuracy, specificity, PPV, and 97.5% sensitivity. At the genus level, their agreement was 0.979, and at the species level, it was 0.958. The gyr B gene appeared in all 40 A. baumannii isolates. The most effective antibiotic was trimethoprim-sulfamethazole (37.5%) for acinetobacter infections. Biofilm formation occurred in 52.5% of cases via plate and tube methods. **Conclusion:** PCR and BD Phoenix M50 are rapid, specific, reliable and easy to interpret methods for accurate detection of Acinetobacter baumannii.

Keywords: Rapid Identification; Acinetobacter baumannii; ICU Patients.

1. Introduction:

Acinetobacter is an aerobic, non-fermentative, oxidase-negative, gram-negative, nonmotile microorganism [1].

Although there are other species of Acinetobacter, A. baumanii is the one with the most recognized clinical importance. Acinetobacter must be identified precisely because these infections differ in their biology and clinical traits. Furthermore, these pathogens have varying capacities in terms of skin colonisation, susceptibility to antibiotics, and mechanisms of resistance to antibiotics [2].

In many hospitals, Acinetobacter baumannii is a significant contributor to nosocomial infections. Because of its strong environmental tolerance and propensity to become resistant to antibiotics, it is challenging to control and cure infections caused by it [3].

Nosocomial pneumonia, particularly "late-onset" pneumonia associated with ventilator usage, is frequently caused by A. baumannii. A. baumannii can live on human skin or dry surfaces for weeks at a time, and it can cause a number of different illnesses, such as bacteremia, meningitis, and so on [4].

Acinetobacter is a low-virulence organism, but it is highly transmissible in a hospital setting because of its capacity to withstand desiccation and last for long periods of time in the environment. There have been numerous reports of nosocomial dissemination by medical staff, breathing equipment including ventilators, and other devices. It is present in all kinds of secretions, including blood, urine, saliva, and wounds [5].

An essential virulence mechanism and distinguishing feature of A. baumannii is biofilm development. The formation and maintenance of A. baumannii biofilms are facilitated by a variety of microbial features, including adhesins, capsular polysaccharides, surface appendages, virulence genes, and resistance determinants, as well as physicochemical factors, such as temperature, growth media, surface hydrophobicity, pH, and oxygen concentration, and other factors like biofilm-associated protein (Bap) and the outer membrane protein A (OmpA) [6].

Because A. baumannii exhibits widespread resistance to drugs and high molarity mortality in conjunction with its infections, these illnesses have been deemed to be of great concern. Multiple antibiotic resistance mechanisms can be developed by A. baumannii strains, which poses a serious risk to the health of immunocompromised patients. These bacteria displayed resistance to third generation cephalosporins, carboxy-pencillins, and the most recent generation of carbapenems, which are broad-spectrum β-lactam antibiotics. Moreover, these strains are able to manufacture a variety of aminoglycoside-modifying enzymes, the majority of which are connected to fluoroquinolone resistance [7].

Because it is very selective and simple to use, gyrB gene analysis is a promising method for differentiating between ABC complex species [8].

The aim of this work was to evaluate the efficiency of polymerase chain reaction for early, rapid and reliable diagnosis of Acinetobacter baumannii among ICU patients, detect A. baumannii's biofilm production isolates and to determine the antimicrobial susceptibility pattern among A. baumannii isolated from ICU patients.

2. Methods:

This cross-sectional study involved 40 isolates of Acinetobacter baumannii bacteria from patients who developed clinical evidence of nosocomial infection. From May 2020 to June 2021, a whole year was dedicated to conducting the study. Different types of

specimens were collected as follows: Lower respiratory tract: 25 specimens were collected: A- In non-intubated patients: morning sputum samples were collected in a sterile cup and B- In intubated patients: samples were taken by suction catheter through mini bronchoalveolar lavage. Urine: 3 samples were collected: A- In non-catheterized patients: mid-stream urine specimens were obtained aseptically in sterile containers, and B- In catheterized patients: samples were collected as follows: Part of the catheter was cleaned with antiseptic solutions and needle of a syringe was inserted to aspirate 2-4 ml fresh urine in a sterile container [8]. Wound: 2 samples were collected from infected surgical and traumatic wounds. The wound surface was cleaned with sterile saline and without using a germicidal agent to irrigate any purulent debris, as much of the surface flora as possible should be removed and to achieve a clean culture site [8]. Blood: 10 samples were collected from peripheral vein by venipuncture or central venous line through the distal lumen [8]. A blood culture specimen was drawn at one time from one location, in order to maximize the possibility of a true-positive result, and prior to the prescription of antibiotics when the patient exhibits signs and symptoms of a bloodstream infection, such as fever or chills. In less than a day, the patient had stopped taking antibiotics [8].

Each and every patient underwent full history taking, (patient's name, age, gender, duration of hospitalization, presence of chronic disease, use of invasive device and history of antibiotics intake.).

All Samples were **Cultured on conventional culture media**: Cultured on blood agar medium as a non-selective enrichment medium & MacConkey agar medium as a differential and selective one. Cultures underwent an aerobic 18–24-hour incubation at 37°C. Blood samples collected under complete aseptic condition in specific blood culture bottles and monitored for microbial development within the Bact alert 3D apparatus (Biomerieux, France). Agar plates were used to subculture the bottles that the Bact-alert system had identified as positive.

The isolated colonies were identified by conventioal methods: 40 isolates were suspected to be Acinetobacter species by: **Colony appearance**: Acinetobacter colonies were smooth, convex, glossy, and occasionally mucoid. They were pale yellow, non-lactose fermenters, and appeared as grayish-white colonies on blood medium and 2-3 mm in diameter on MacConkey media. It did not lyse blood. **Gram staining:** Acinetobacter were initially short, rod-shaped, Gram-negative bacteria, although they were frequently more coccoid and found in pairs or clusters. **Biochemical reaction:** [8] Acinetobacter bacteria were Oxidase test negative, Catalase positive, non-motile by motility test (MIO), In Triple sugar test, it is k/k, LIA test, it is k/k, Urease test are negative while citrate test is almost positive.

Identification by automated methods: for the identification of Acinetobacter at the species level. BD Phoenix™ M50 (**Becton, Dickinson and company, USA)**. The Automated Microbiology System offers great accuracy and quick turnaround times because to its innovative susceptibility testing technology. BD Phoenix panels **(Becton, Dickinson and company, USA)** offer precise, quick identification and susceptibility data for the majority of therapeutically relevant facultative anaerobic and aerobic Gram-negative and Gram-positive bacteria.

Biofilm formation: The ability of A. baumannii isolates to form biofilms was evaluated using the tube method and 96-well microtiter plates. At the air-liquid interface, movable microorganisms usually form a biofilm on the walls, bottoms, and/or surfaces of the tubes and wells.

Microtiter plates: After being separated from fresh agar plates, the organisms were injected into 10 millilitres of Trypticase soy broth (TSB) containing 1% glucose and allowed to incubate for 24 hours at 37°C. Thereafter, the cultures were diluted 1:100. Two hundred microliters of diluted cultures were placed in each well of a sterile 96-well flat-bottom polystyrene tissue culture treatment plate. There was sterile broth in the negative control wells. The plates were incubated for 24 hours at 37°C. Following incubation, the contents of each well were carefully tapped out. Four times, 200 µl of phosphate buffer saline (PH 7.2) was used to wash the wells. Bacteria that adhered to the wells generated biofilm, which was fixed by 2% sodium acetate for 20 minutes and stained by 0.1% crystal violet for 15 minutes. Utilizing a Pasteur pipette, the microwell plastic plate was cleaned three times with tap water before being allowed to air dry at room temperature. Tests were conducted on motile bacteria that stuck to the wells' bottoms or walls [8].

Tube method (TM); is a qualitative method for identifying microorganisms that generate biofilms: The isolates were inoculated into a test tube containing 10 millilitres of trypticase soy broth with 1% glucose, and the tube was then incubated at 37°C for 24 hours. Following incubation, the tubes were dried and cleaned with phosohate buffer saline (pH: 7.3). Crystal violet discoloured the tubes (0.1%). Deionized water was used to remove any excess stain. The tinted tubes were then turned inside out and allowed to dry. In positive samples, there was evidence of visible film [8].

Molecular identification by: PCR for identifying specific sequence of A. baumannii (gyrB gene sequences).

The gyrB gene sequences from A. baumannii isolates were aligned, and three primers were created, two of which were common to all Acinetobacter species, sp4F (5`CACGCCGTAAGAGTGCATTA) and sp4R (5`AACGGAGCTTGTCAGGGTTA), and one differed at the 3` end which is specific to acinetobacter baumannii, sp2F (5`GTTCCTGATCCGAAATTCTCG). Gradient PCR was performed to determine the optimum annealing temperature for sp2F so that it would produce a PCR product with A. baumannii, only. thus, in a PCR with all three primers, only A. baumannii would yield

the two amplicon; the first amplicon of 294 bp (sp4F to sp4R) and a second amplicon of 490 bp (sp2F to sp4R).

The selected samples were subjected to DNA extraction using QIAamp® DNA Mini and Blood axtraction kit. **(Qiagen, Germany)**.

DNA amplification was performed in 50 µl final reaction volumes with 25 µl COSMO Hot Start'PC RED Master Mix (**Willowfort, United Kingdom**), primer concentrations of 0.2µM for each **(Eurofins, Germany)** and 200 ng DNA template and nuclease-free sterile water to adjust the reaction volum.

Conditions of the thermal cycler (**T100 Thermal Cycler (BIO RAD, USA**)) for PCR was the following: 95 °C for 2 minutes, then 30 cycles of 95 °C for 10 seconds, 52 °C for 20 seconds, and 72 °C for 1 minute, concluding with a final extension at 72 °C for 1 minute. PCR products were analyzed on 2% agarose gels and visualized on a UV transilluminator. Amplified gene was identified on the basis of fragment size using 100bp plus DNA Ladder. Positive gyr B gene fragment was 294 bp and 490bp.

Statistical analysis:

The Statistical Package for Social Science was utilized to analyze the data that was gathered. (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Shapiro test was used as a test of normality. In non-parametric numerical data, median and range are found. Frequency and percentage of non-numerical data. The statistical significance of the difference in a non-parametric variable between two research groups was determined utilizing Mann Whitney Test (U test). The statistical significance of the difference between more than two study group non-parametric variables was determined utilizing Kruskal-Wallis test. Chi-Square test was utilized to examine the relationship between two qualitative variables. The kappa statistic was calculated to estimate agreement between the methods. A p value is considered significant if <0.05 at confidence interval 95%.

3. Results:

This study was carried out on 40 A. baumannii isolates from 40 ICU patients ;21 female and 19 males with age ranged between 2 weeks – 93 years, the mean age was 46.5 (0.02-93) years. No sex predominance was noticed while in concerning with associated comorbidities, hypertension and diabetes mellitus were predominant 42.5% and 37.5% respectively. **Table 1**

Table (1) Demographics data, ICU admission, comorbidities, external devices, and types of samples among all studied cases

		Patients (n = 40)
Age (years)		46.5 (0.02-93)
Adult		30(75%)
Pediatric		5(12.5%)
Neonates		5(12.5%)
Sex	**Male**	21(52.5%)
	Female	19(47.5%)
ICU admission	**Adult ICU**	30(75%)
	Pediatric ICU	5(12.5%)
	Neonates ICU	5(12.5%)
Comorbidities	**No comorbidities**	15(37.5%)
	Comorbidity	25(62.5%)
	DM	15(37.5%)
	Hypertension	17(42.5%)
	Cardiac disease	3(7.5%)
	Hepatic disease	2(5.0%)
	Lung disease	2(5.0%)
External device	**Peripheral venous line**	24(60.0%)
	Urinary catheter	14(35.0%)
	Central venous line	10(25.0%)
	Chest tube	8(20.0%)
Sample	**Sputum**	25(62.5%)
	Blood	10(25.0%)
	Urine	3(7.5%)
	Wound (Pus)	2(5.0%)

Data are presented as Median (range) or frequency (%), DM: Diabetes Mellitus.

No significant association was found regarding prognosis with age and gender, comorbidities, different types of samples and length of hospitalization.

Patients with CVP were significantly associated with higher mortality rates (p=0.35). Otherwise, no significant association was found regarding prognosis with Peripheral venous line. **Table 2**

Table 2: Association of prognosis with age, gender comorbidities, external devices, types of samples and length of hospitalization

		Prognosis				
		Cure		Death		P
		N=30		N=10		
Age (years)		47	(0.02-93)	42.5	(10-69)	0.767
Adult		22	73.3%	8	80.0%	0.148
Pediatric		4	13.3%	1	10.0%	
Neonates		4	13.3%	1	10.0%	
Sex	**Males**	18	60.0%	3	30.0%	0.915
	Females	12	40.0%	7	70.0%	
Comorbidities	**No comorbidity**	11	36.7%	4	40.0%	0.850
	Yes	19	63.3%	6	60.0%	
	DM	10	33.3%	5	50.0%	0.457
	Hypertension	14	46.7%	3	30.0%	0.471
	Cardiac disease	1	3.3%	2	20.0%	0.149
	Hepatic disease	2	6.7%	0	0.0%	0.402
	Lung disease	2	6.7%	0	0.0%	0.402
Device	**Peripheral venous line**	16	53.3%	8	80.0%	0.263
	Urinary catheter	11	36.7%	3	30.0%	0.702
	Central venous line	5	16.7%	5	50.0%	0.035*
	Chest tube	5	16.7%	3	30.0%	0.388
Sample	**Sputum**	19	63.3%	6	60.0%	0.723
	Blood	6	20.0%	4	40.0%	
	Urine	3	10.0%	0	0.0%	
	Pus	2	6.6%	0	0.0%	
Length of hospitalization (days)		11(0-40)		10(2-30)		0.584

Data are presented as Median (range) or frequency (%). DM: diabetes mellitus – HTN: hypertension.

PCR had perfect agreement with culture for detection of acinetobacter (k=1). PCR has sensitivity of 100%, specificity of 100%, PPV of 100%, NPV of 100, accuracy of 100%. Phoenix had excellent agreement with culture for detection of acinetobacter (0.979). Phoenix has sensitivity of 97.5%, specificity of 100%, PPV of 100%, NPV of 98.4, accuracy of 99%. **Table 3**

Table 3: Validity of PCR, and BD phoenix™ M50 in detection of Acinetobacter species in comparison to culture as gold standard

Culture		Kappa	Agreement	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
	PCR	1	Perfect	100	100	100	100	100
	Phoenix	0.979	Excellent	97.5	100	100	98.4	99

No significant association was found regarding biofilm formation with age, gender, comorbidities, types of devices, different types samples, and the length of hospital stay. **Table 4**

Table 4: PCR, BD phoenix™ M50 results, Biofilm formation of the selected samples and association of biofilm formation with age, gender, comorbidities, devices, samples, and outcome

	Samples (n=40)				
	Count		Kappa		
PCR (species level)	40		1		
Phoenix (species level)	38		0.958%		
Biofilm	**Absent**		**Present**		**P**
	N=19		**N=21**		
Age (years)	37(0.03-73)		48 (0.2-93)		0.766
Adult	14	73.7%	16	76.2%	0.882
Pediatric	2	10.5%	3	14.3%	
Neonates	3	15.8%	2	9.5%	
Males	9	47.4%	12	57.1%	0.536

Females		10	52.6%	9	42.9%	
Comorbidities	**No**	6	31.6%	9	42.9%	0.462
	Yes	13	68.4%	12	57.1%	
	DM	8	42.1%	7	33.3%	0.567
	HTN	6	31.6%	11	52.4%	0.184
	Cardiac	2	10.5%	1	4.8%	0.489
	Hepatic	2	10.5%	0	0.0%	0.127
	Lung diseases	2	10.5%	0	0.0%	0.127
Device	**Peripheral venous line**	13	68.4%	11	52.4%	0.301
	Urinary catheter	7	36.8%	7	33.3%	0.816
	Central venous line	6	31.6%	4	19.0%	0.473
	Chest tube	4	21.1%	4	19.0%	0.874
Sample	**Sputum**	12	63.1%	13	61.9%	0.959
	Blood	5	26.3%	5	23.8%	
	Urine	2	10.5%	1	4.8%	
	Wound	0	0	2	9.6%	
Length of hospitalization (days)		0 (0-40)		15 (2-30)		0.221
Prognosis	**Cure**	14	73.7%	16	76.2%	0.855
	Death	5	26.3%	5	23.8%	

Data are presented as Median (range) or frequency (%).

No significant association was found regarding biofilm formation with the pattern of resistance. **Table 5**

No significant association was found regarding biofilm formation with susceptibility patterns.

Table 5: Susceptibility patterns among the 40 selected isolates, resistance pattern by the automated BD Phoenix™ M50 and association of biofilm formation with the pattern of resistance.

		Samples n=40					
		Sensitive		**Intermediate**		**Resistant**	
Amikan		5	12.5%	0	0%	35	87.5%
Gentamycin		0	0%	0	0%	40	100%
Ertapenem		0	0%	0	0%	40	100%
Imipenem		8	20%	1	2.5%	31	77.5%
Meropenem		0	0%	0	0%	40	100%
Cefazoline		0	0%	0	0%	40	100%
Cefuroxime		0	0%	0	0%	40	100%
Ceftazidime		0	0%	0	0%	40	100%
Ceftriaxone		0	0%	0	0%	40	100%
Cefepime		0	0%	0	0%	40	100%
Ampicillin		0	0%	0	0%	40	100%
Amoxicilline -clavulanate		0	0%	0	0%	40	100%
Piperacillin -tazobactam		0	0%	0	0%	40	100%
Trimethoprim – sulfamethazole		15	37.5%	0	0%	25	62.5%
Nitrofurantoin		0	0%	0	0%	40	100%
Ciprofloxacine		6	15%	0	0%	34	85%
Levofloxacine		6	15%	0	0%	34	85%
Colistin *		13	-	-	-	1	-
Tigecycline *		-	-	-	-	-	-
		Positive by Phoenix n=39					
		Count		**%**			
Resistance	**None**	10		25.6%			
	CARB	27		69.2%			
	CARB and B. lactamase	2		5.1%			
		Biofilm					
		Absent		**Present**		**P**	
		N=19		**N=21**			
Resistance	**None**	6	31.6%	5	23.8%	0.583	
	Résistance	13	68.4%	16	76.2%		
	CARB	12	63.2%	15	71.4%	0.859	
	CARB and B. lactamase	1	5.3%	1	4.8%		

Data are presented as Median (range) or frequency (%), CARB: Carbapenem resistance. *Colistin and tigecycline: Colistin resistance of A. baumannii is greatly underestimated by semiautomated system, potentially leading to inappropriate colistin administration.

4. Discussion:

Acinetobacter species, particularly A. baumannii, are among the recently discovered bacteria worldwide. Apart from the respiratory tract infections and bacteremia caused by A. baumannii, the bacterium can also cause infections of the urinary tract, secondary meningitis, peritonitis, and other types, particularly in patients with impaired immune systems or those undergoing invasive operations [9].

The present study was conducted on 40 acinetobacter baumannii cases selected from 200 ICU patients in different departments. In our study among selected cases with Acinetobacter baumannii infected, their age ranged from 2 weeks to 93 years; 75% were adults, 12.5% were pediatrics and 12.5% were neonates. This was in agreement with **Gupta et al** study, that showed more acinetobacter infection in age group >50 years (31 [28%]) [10], 62.5% had comorbidities, hypertensive was the most prevalent underlying illness, which 42.5% of patients had, followed by DM 37.5%, while 7.5% were cardiac, 5% were hepatic and 5% had underlying lung diseases. **Chiang et al.** [10] revealed that the majority of the patients were severely ill and had at least one underlying disease. The most frequent underlying systemic disease, discovered in 74.4% of the patients, was cardiovascular disease.

Among all studied cases, 60% had peripheral venous line; 35% had urinary catheter, 25% had central venous line and 20% had chest tube. Median Length of hospitalization was 11 days, ranged from 0 to 40 days. This result was in disagreement with **Patel et al.** [11] that showed that central venous that central (VCs) were the most common invasive devices.

The current study's findings demonstrated that the bulk of A. baumannii isolates came from sputum samples (62.5%). In agreement with our study, **Mezzetesta et. al.** [10] discovered that sputum samples contained 70.1% of Acinetobacter species.

The clinical spectrum may vary depending on the source, the use of antibiotics, the severity of the underlying illness, the hospital setting, the length of the patient stays, and the infection control procedures used in various hospitals [12].

The present study revealed that most of studied cases cured (75%), while 25% died. In cordance with the findings in current study, the study done by **Xiao et al.** found that ICU mortality rate: 21.6% [13] and lower than 45% mortality rate was found in study done dy **patel et al.** [12].

BD Phoenix™ M50 had excellent agreement with culture for detection of Acinetobacter species (0.979). BD Phoenix possesses 99% accuracy, 100% specificity, 100% PPV, 98.4 NPV, and 97.5% sensitivity.

Diagnostic validity test was done for PCR taking culture as a gold standard test and we found that PCR had perfect agreement with culture for detection of A. baumannii (k=1). PCR offers 100% accuracy, 100% NPV, 100% PPV, 100% specificity, and 100% sensitivity. In line with our finding **McConnell et al.** [14] showed that surfaces infected with A. baumannii can be quickly and highly sensitively recognised by real-time PCR (100%).

Unlike our study, **Rabaan et al.** [15] showed that by standard culture, two PCR-positive samples turned out to be negative. The percentage of concordance overall was 99.5%, while the percentage of positive concordance was 85.7%.

Out of 40 acinetobacter samples, phoenix identified 39 and one sample was missed (when the comparison is made with culture). Phoenix had excellent agreement with culture for detection of acinetobacter species (0.979). Phoenix has sensitivity of 97.5%, specificity of 100%, PPV of 100%, NPV of 98.4, accuracy of 99%.

At the species level of identification, according to our study; PCR detect 40 positive samples as A. baumannii and Phoenix detects 38 positive samples as A. baumannii and 1 sample was misidentified as Acinetobacter lowffii.

The agreement at the genus level of identification was 0.979 while the agreement at species level was 0.958.

The present study showed that biofilm was formed in 52.5% by microtitre plate and tube methods. These outcomes are in line with earlier research from other studies that discovered values ranging from 50% to 76% globally [16-19].

Moreover, we found no significant association was found regarding biofilm formation with age, gender, comorbidities, devices, sample type, phonix results, susceptibility patterns and outcome. Similarly, **Asaad et al.** showed that regarding the clinical features (kinds of specimen, comorbidities, ward admission, or invasive procedures), there were no significant differences found between A. baumannii isolates that formed biofilms and those that did not.

There are several ways that A. baumannii's carbapenem sensitivity is weakened. The most prevalent mechanism by far is carbapenemases [20].

Regarding antibiotic susceptibility testing using BD Phoenix™ M50 in our study, 25.6% revealed no resistance, 69.2% revealed CARB, and 5.1% revealed CARB and B. lactamase. This was matched by **Fattouh and Nasr El-Din** [21] in Sohag who reported that 71.4% of Acinetobacter isolates were imipenem resistant.

Moreover, we found that susceptibility patterns among all studied samples; trimethoprim – sulfamethazole showed highest sensitivity pattern (37.5%). While most of studied samples were resistant to all isolated organisms as gentamycin, ertapenem, meropenem, meropenem, cefazoline, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin,

amoxicilline –clavulanate, piperacillin –tazobactam, nitrofurantoin.

Our study reveals (100%) resistance to Acinetobacter for: cefepime, piperacillin–tazobactam, ceftazidime, ciprofloxacin, (87.5%) amikan and (77.5) imipenem. Similarly, the study done by **Fouad et. al.,** [22] in Cairo found high resistance rate of Acinetobacter spp. to antibiotics: 100% for each of the following: amikacin, aztreonam, gentamicin, tobramycin, and ceftazidime, cefepime, ciprofloxacin, and piperacillin/tazobactam. This also was matched with the outcomes of **Baker et al.** [23] who revealed high rates of resistance to ampicillin–sulbactam, cefepime (91% for each), piperacillin, ceftazidime (88.5% for each), piperacillin–tazobactam (84.6%), tobramycin (80.8%), amikacin, and tetracyclines (75.6% for each).

As regarding relation of biofilm producing isolates with antibiotic resistance, we found no significant association although higher resistance rates to amikan, imipenem, trimethoprim-sulphamethazole, ciprofloxacine and levofloxacine (p = 0.345, 0.241, 0.935, 0.664 and 0.664 respectively). Opposing our results **Asaad et. al.** [24] discovered that the biofilm-forming isolates had a statistically significant increased resistance rate to imipenem, trimethoprim/sulfamethoxazole, gentamycin, ampicillin/sulbactam, piperacillin/tazobactam, and ceftazidime (p = 0.041, <0.001, 0.006, 0.034, 0.028, 0.002, 0.002, and 0.02, respectively).

It is necessary to quickly and accurately identify strains that are resistant to polymyxins because this resistance has presented a significant problem in the treatment of infectious diseases. The broth microdilution (BMD) method is the accepted reference method for colistin (polymixine E) antimicrobial susceptibility testing (AST); however, it is seldom carried out manually in clinical practise due to the laborious nature of the process and the stringent testing requirements [22].

In our study, there was an error in the performance of automated system (Phoenix) for colistin and tigecycline. similarly, study done by **Zhu Y, et. al.** [22] found that, when used in place of the gold standard test, the broth microdilution, to determine polymyxin susceptibilities (colistin), VITEK 2 R COMPACT (BioMérieux) and PhoenixTM M50 (Becton Dickson Diagnostics) perform unsatisfactorily because none of these systems met the standards for colistin and polymyxin B AST (BMD). On the contrary, **Fouad et al.** [22] had satisfactory results as they found that 56.4% of Acinetobacter spp. were susceptible to tigecycline. **Behera et al.** [22] and **Koripella et al.** [22] in India exhibited that 42 and 91.3% of isolated Acinetobacter, respectively were tigecycline sensitive.

5. Conclusions:

Culture is accurate and cheap method for diagnosis of acinetobacter bacteria but not at species level. In our study, PCR had perfect agreement with culture for detection of acinetobacter (k=1) and sensitivity of 100%, specificity of 100% PPV of 100%, NPV of 100, accuracy of 100%. PCR gives great results as accurate, rapid and specific method but it is relatively expensive.

BD Phoenix™ M50 had excellent agreement with culture for detection of Acinetobacter species (0.979). BD Phoenix has sensitivity of 97.5%, specificity of 100%, PPV of 100%, NPV of 98.4, accuracy of 99%. The agreement between PCR and Phoenix at the genus level of identification was 0.979 while the agreement at species level was 0.958. So, the agreement between studied methods ranged from 0.958 to 1 (excellent to perfect).

Biofilm formation was found in more than 50 % of isolates. We didn't found relation between biofilm formation and comorbidity, but it shouldn't be generalized and further investigation on wide scale should be done. Nowadays, A. baumanni is considered amultidrug resistance organism it developed resistance to most of antibiotics.

References

[1] 1. T. Wang, V. Costa, S.G. Jenkins, B.J. Hartman, L.F. Westblade. Acinetobacter radioresistens infection with bacteremia and pneumonia. IDCases;15:e00495. 2019

[2] 2. S.S. Abhari, O. Azizi, L. Modiri, M.M. Aslani, M. Assmar, S. Fereshteh, et al. Two new rapid PCR-based methods for identification of Acinetobacter baumannii isolated from clinical samples. Mol Cell Probes;58:101732. 2021

[3] 3. J.M. Cisneros, J. Rodríguez-Baño. Nosocomial bacteremia due to Acinetobacter baumannii: epidemiology, clinical features and treatment. Clinical Microbiology and Infection;8:687-93. 2002

[4] 4. J.M. Cisneros-Herreros, J. Garnacho-Montero, M.E. Pachón-Ibáñez. [Nosocomial pneumonia due to Acinetobacter baumannii]. Enferm Infecc Microbiol Clin;23 Suppl 3:46-51. 2005

[5] 5. F. de Azevedo, V. Dutra, L. Nakazato, C.M. Mello, M.A. Pepato, A. de Sousa, et al. Molecular epidemiology of multidrug-resistant Acinetobacter baumannii infection in two hospitals in Central Brazil: the role of ST730 and ST162 in clinical outcomes. J Med Microbiol;68:31-40. 2019

[6] 6. S. Roy, G. Chowdhury, A.K. Mukhopadhyay, S. Dutta, S. Basu. Convergence of Biofilm Formation and Antibiotic Resistance in Acinetobacter baumannii Infection. Front Med (Lausanne);9:793615. 2022

[7] 7. S. Ibrahim, N. Al-Saryi, I.M.S. Al-Kadmy, S.N. Aziz. Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals. Mol Biol Rep;48:6987-98. 2021

[8] 8. G.M. Ederer, M. Clark. Motility-indole-ornithine medium. Appl Microbiol;20:849-50. 1970

[9] 9. X. Cheng, J. Yang, M. Wang, P. Wu, Q. Du, J. He, et al. Visual and rapid detection of Acinetobacter baumannii by a multiple cross

displacement amplification combined with nanoparticles-based biosensor assay. AMB Express;9:30. 2019

[10] 10. M.-C. Chiang, S.-C. Kuo, Y.-C. Chen, Y.-T. Lee, T.-L. Chen, C.-P. Fung. Polymerase chain reaction assay for the detection of Acinetobacter baumannii in endotracheal aspirates from patients in the intensive care unit. Journal of Microbiology, Immunology and Infection;44:106-10. 2011

[11] 11. R.V. Patel, J.S. Shah, G. Revathi, W. Siika, R. Shah. Acinetobacter infections: a retrospective study to determine inhospital mortality rate and clinical factors associated with mortality. Infect Prev Pract;1:100010. 2019

[12] 12. C. Tsioutis, E.I. Kritsotakis, S.A. Karageorgos, S. Stratakou, C. Psarologakis, S. Kokkini, et al. Clinical epidemiology, treatment and prognostic factors of extensively drug-resistant Acinetobacter baumannii ventilator-associated pneumonia in critically ill patients. Int J Antimicrob Agents;48:492-7. 2016

[13] 13. D. Xiao, L. Wang, D. Zhang, D. Xiang, Q. Liu, X. Xing. Prognosis of patients with Acinetobacter baumannii infection in the intensive care unit: A retrospective analysis. Exp Ther Med;13:1630-3. 2017

[14] 14. M.J. McConnell, A. Pérez-Ordóñez, P. Pérez-Romero, R. Valencia, J.A. Lepe, I. Vázquez-Barba, et al. Quantitative real-time PCR for detection of Acinetobacter baumannii colonization in the hospital environment. J Clin Microbiol;50:1412-4. 2012

[15] 15. A.A. Rabaan, J.V. Saunar, A.M. Bazzi, W.F. Raslan, D.R. Taylor, J.A. Al-Tawfiq. Epidemiology and detection of acinetobacter using conventional culture and in-house developed PCR based methods. Journal of Infection and Public Health;10:124-8. 2017

[16] 16. A. Bahador, Z. Farshadzadeh, R. Raoofian, M. Mokhtaran, B. Pourakbari, M. Pourhajibagher, et al. Association of virulence gene expression with colistin-resistance in Acinetobacter baumannii: analysis of genotype, antimicrobial susceptibility, and biofilm formation. Ann Clin Microbiol Antimicrob;17:24. 2018

[17] 17. H. Zeighami, F. Valadkhani, R. Shapouri, E. Samadi, F. Haghi. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. BMC Infect Dis;19:629. 2019

[18] 18. R.H. Sunenshine, M.O. Wright, L.L. Maragakis, A.D. Harris, X. Song, J. Hebden, et al. Multidrug-resistant Acinetobacter infection mortality rate and length of hospitalization. Emerg Infect Dis;13:97-103. 2007

[19] 19. D.J. Weber, W.A. Rutala, M.B. Miller, K. Huslage, E. Sickbert-Bennett. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control;38:S25-33. 2010

[20] 20. J.M. Pogue, T. Mann, K.E. Barber, K.S. Kaye. Carbapenem-resistant Acinetobacter baumannii: epidemiology, surveillance and management. Expert Rev Anti Infect Ther;11:383-93. 2013

[21] 21. M. Fattouh, A.N. El-Din. Emergence of carbapenem-resistant Acinetobacter baumannii in the intensive care unit in Sohag university hospital, Egypt. International Journal of Current Microbiology and Applied Sciences;3:732-44. 2014

[22] 22. Y. Zhu, P. Jia, M. Zhou, J. Zhang, G. Zhang, W. Kang, et al. Evaluation of the Clinical Systems for Polymyxin Susceptibility Testing of Clinical Gram-Negative Bacteria in China. Front Microbiol;11:610604. 2020

[23] 23. A. Baker, A. Makled, E. Salem, A. Salama, S. Ajlan. Phenotypic and molecular characterization of clinical Acinetobacter isolates from Menoufia University Hospitals. Menoufia Medical Journal;30:1030-6. 2017

[24] 24. A.M. Asaad, S. Ansari, S.E. Ajlan, S.M. Awad. Epidemiology of Biofilm Producing Acinetobacter baumannii Nosocomial Isolates from a Tertiary Care Hospital in Egypt: A Cross-Sectional Study. Infect Drug Resist;14:709-17. 2021