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Isolation and Evaluation of Clinically Important Acinetobacter Baumannii From Intensive Care Unit Samples

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Article Info.	Abstract
Article history:	Acinetobacter baumannii is considered a critical healthcare problem for patients in intensive care units due to its high ability to be multidrug-resistant to most commercially available antibiotics. The current study is at aimed at isolating and identifying the clinical isolates of A. baumannii from different samples and investigating the antibiotic resistance of
Received 03 May 2021	isolates. Isolation and diagnosis of bacteria were achieved by conventional techniques, including routine and selective culture media (Chrome agar), biochemical test, EPI 20E, and VITEK-2. These methods were basically considered as a gold standard for identification of A. baumannii infections from different clinical sources, and culture positive isolates
Accepted 11 September 2021	were tested for antibiotic susceptibility using a modified Kirby–Bauer method. A total of 375 clinical specimens were collected from different infections in some hospitals in Mosul and Erbil cities/Iraq from Sep 2020 to Jan 2021. Overall, 41 isolates were identified as A. baumannii using conventional and biochemical methods, and then confirmed by VITEK-2
Publishing 30 September 2021	system. Our results established that only 41(14.4%) isolates were diagnosed as A. baumannii, and most of these isolates were from burns (36.5%), surgical wounds (34.1%), and sputum (14.6%). However, it was identified in CSF, blood, and urine samples with lower percentages (7.3%, 4.8%, and 2.4%, respectively). The clinical isolates of A. baumannii showed high to moderate resistance to Piperacillin (97.5%), Piperacillin/Tazobactam., Ceftazidime (87.8%), Meropenem (85.3%), Tri/slphamethoxazole (82.9%), Levofloxacin (80.4%), Imipenem, Ciprofloxacin (78%), Gentamycin (75.6%), Amikacin (73.1%), Netilmicin (68.2%), Tobramycin (60.9%) and Tetracycline (31.7%). However, two antimicrobial agents which were Colistin and Tigecycline produced 0 and 2.4 % resistance to A. baumannii respectively, which were considered the most used choices to treat A. baumannii infections. The current findings suggest that automated Vitek 2 system is the most common method to accurately detect the isolates and evaluate multi-drug resistant A. baumannii among patients.

Keywords: Acinetobacter baumannii; VITEK-2; Antibiotic susceptibility; Intensive Care Unit

1. Introduction

Acintobacter baumannii (A. baumannii) is a non-fermenting coccobacilli; oxidase negative, aerobic bacilli and human opportunistic extracellular pathogen originating from hospital acquired infections to be known also as a nosocomial infection [1-2]. Most of A. baumannii bacteria were isolated from the patients of ICU who used mechanical ventilation and catheterization, which are considered risk factors that lead to biofilm formation in A. baumannii infection [3]. Surprisingly, hospital furniture such as patients' mattresses, bed railings, curtains, stethoscopes, computers, and telephones were the main sources of A. baumannii infection, where this bacterium was isolated [4]. Therefore, there is an extreme need to prevent A. baumannii infections especially in ICU by creating new strategies and managements for isolation [5]. Acinetobacter species caused many diseases such as pneumonia, bacteremia, UTIs, skin and soft tissue infections leading to high escalation of morbidity and mortality [6]. A. baumannii was collected from different sources such as, damaged skins and soft tissues with second and third burn grades. Furthermore, an American study on injured soldiers showed that A. baumannii was frequently isolated from serious injuries for instance, open tibia fractures which particularly occurred in the wars accidents of Iraq and Afghanistan [7].

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Symbols			
API 20 E	(Analytical Profile Index) 20 Enterobacteriaceae	ID-GNB	Identification card gram negative bacilli
CSF	Cerebrospinal fluid	TDA	Tryptophan Deaminase Reagent
ICU	Intensive care unit	°C	Celsius Degree
UTI	Urinary tract infection	P. value	probability value
MDR	Multi drug resistance	G-ve	Gram negative bacteria
XDR	Extensive drug-resistant	G+ve	Gram negative bacteria
PDR	Pandrug-resistant	AST	Antibiotics susceptibility test
EM	Emergency management center	X2	Chi square
BHI	Brain hard fusion	VP	Voges-Proskauer

A. baumannii infections cause many diseases and this created a significant concern around the world. One of the most common phenomena that support A .baumannii for survival and resistance to most antibiotics is biofilm formation, which causes contamination of medical equipment[2]. The resistance of bacterial biofilms is not only to antibiotics but also develop to chemicals, phagocytosis, and other components of the innate and acquired immune system of the body. Consequently, there is a necessary need to create a connection between biofilm formation and antibiotics resistance in isolates of A. baumannii [4]. Conventional detections were used for early diagnosis to distinguish the infections of this organism in the hospitals, which considered as a routine cultivation methods [5-6]. This bacterium can be transmitted through exposure to aerosol droplets, hands touching from person-to-person, sputum, urine, faces or via surfaces contaminated with fomite in the hospitals [7]. A. baumannii's created critical healthcare problems for patients in ICU ward due to its ability to have high prevalence of multidrug resistant (MDR) to most of antibiotics commercially available [8-9]. This paper will highlight the isolation and identification of A. baumannii from different samples such as burns, wounds, blood, sputum, Cerebrospinal fluid (CSF) and urine of inpatients in Erbil and Mosul hospitals using conventional methods, in addition to, the investigation of the susceptibility of A. baumannii isolates to different antimicrobial agents.

2. Materials and Methods

2.1. Collection of clinical samples

This study was designed to isolate A. baumannii from different clinical samples. All patients admitted to ICU wards were aged between 5 months to 81 years from both males and females. The total collected samples are (375), from three various hospitals in Erbil city (Emergency Management Centre (EMC), Par Hospital and Virkary Children's Educational hospitals) and two hospitals in Mosul city (Al-Jumhori Teaching Hospital / Burns ward plastic Surgery and Al-Salam Teaching Hospital) within the period of five months between Sep 2020 to Jan 2021. All specimens were collected from hospitalized patients at the ICU ward from different sources such as urine, blood, CSF, burns, surgical wounds, and sputum. Burns and wounds swabs were carefully taken from the sites of infections, then placed in tubes containing media (Amies with charcoal) to maintain the swabs wet during transferring to be ready for culture. Urine and sputum samples were also collected using sterilized cups and tightly covered. In addition, sterile culture bottles containing brain Heart Infusion broth (BHI) were employed, while CSF samples were carefully collected by an experienced physician. All specimens were labeled and then transported to the lab to register more details for the cultivation process. These samples were collected based on the approval of ethical committees in both Nineveh and Erbil health directorates with gaining consent from the inpatients in ICU wards, where this study was conducted according to the Helsinki Declaration.

2.2. Identification of A. baumannii

All clinical specimens were inoculated initially on MacConkey and blood agars (NEOGEN, USA) and incubated for 24 h at 37°C to identify gram-negative and positive bacteria consecutively. Another selective media (CHROMagar, HiCromeTM, India) was also employed selectively to identify Acinetobacter species [10]. Standard laboratory methods Gram-stain and conventional biochemical methods (Catalase, Oxidase, Citrate utilization test, the reaction in Kligler s iron medium) and Complementary growth at 44°C were also used to identify A. baumannii isolates reconfirmed by using API20E kit [4], VITEK 2 identification system test was also employed using ID-GNB cards, according to the manufacturer's instructions (BioMerieux, France) [11].

2.3. Conventional biochemical tests

Conventional biochemical tests were performed to classify the isolates based on Gram-negative bacterial identification protocols [5] which are mentioned in table 1. 61 . 1

Table 1 The results of biochemical tests of A. baumannii		
Biochemical diagnosis	A. baumannii	
Motility	Non motile	
Fermentative or oxidative	Oxidative	
Oxidase test	Negative	
Catalase test	Positive	
Glucose	Positive	
Xylose	Positive	
Mannitol	Negative	
Sucrose	Negative	

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Galactose	Positive
Manose	Positive
Rhamnose	Positive
Lactose	Positive
Urease test	Negative
Simmons citrate	Positive
Urea, Christensen	Negative
Nitrate reduction	Negative
Methyl red	Negative
Voges-Proskaue	Negative
Kligera iron agar	k/k

2.4. API 20E system

A standardized system which combines several biochemical tests identify a non-fastidious, non-enteric and gram-negative bacilli was performed by Analytical Profile Index (API) 20E [11]. In 2014, A study was achieved by Al Sehlawi to identify *A. baumannii* species by using API 20E according to manufacturer's protocol (BioMerieux) for Enterobacteriaceae and non-enteric bacteria. The process was accomplished by inoculating the wells overnight, 0.5 McFarland bacterial suspensions followed by 24 hrs. incubation at 37°C [12]. The reagents (VP1, VP2), (TDA) and (indole) were respectively added to VP, TDA and Indole tests [10]. After that, the results showed a code number (0204042) and then were compared with reference index of API to confirm that *A. baumannii* identification has been identified[12].

2.5. VITEK[®] 2 system

VITEK[®] 2 is an automated system used to identify the bacterial isolates [11], which basically rely on the biochemical reactions of isolated microorganisms suspended in the solutions and the media of gram negative Card of VITEK[®] 2 system. MacConky agar media was used to inoculate the bacterial isolates followed by incubation for 24 h at 37°C [10]. Samples' preparation was performed by taking a single growth of isolate to be placed in the homogenous suspension solution with an optic density of range (0.55 to 0.63) using a DensiCHEKTM VITEK[®] 2 Caliper [5].

2.6. Antibiotic susceptibility profile

VITEK 2 automated system was also employed to describe the antibiotics of susceptibility profile. A fraction of 145µL of suspended solution of cell, with an optic density between 0.55 and 0.63 was mixed with 3 ml of 0.85% sodium chloride solution. I6 different antibiotics specified of gram negative bacteria were selected, Imipenem (IMP), Meropenem (MEM), Ceftazidime (CAZ), Piperacilin Tazobactam (TZP), Piperacillin (PIP), Tigecycline (TGC) ,Ciprofloxacin (CIP), Levofloxacin (LVE), Gentamicin (G), Tobramycin (TOB), Amikacin (AK), Netaclın, Tetracycline (TE), Trimethoprime-slphamethoxazole (TSX), Colistin (CO), and then analyzed by 2.5 VITEK® 2 system to evaluate the susceptibility profile test [5].

2.7. Statistical analysis

The experimental results were expressed as percentage (%). Statistical analysis was carried out using chi-square. The value of P < 0.05 was considered to be statistically significant. All statistical analyses were carried out using SPSS.

3. Results

3.1. Description and isolation of bacterial species

Culture results showed 297 mixed growths and 78 non-growths. The grown isolates on culture media were only considered in the current study. The bacterial species have been discriminated by gram stain into two groups which are positive and negative bacteria 83 and 214 consecutively. All (214) gram-negative samples have been cultivated on MacConkey agar to obtain a mixed growth of colonies (283) isolates of gram-negative bacteria consisting of (41) *A. baumannii* and (242) of other gram-negative bacteria.

3.2. Distribution of A. baumannii isolates according to the source of clinical samples

In the current study, 41 (14.4 %) isolates of *A.baumannii* were obtained from total isolates of gram-negative bacteria (283). It was found that the highest percentage of recovered *A. baumannii* in burns and wounds with a percentage of (36.5%) and (34.1%) isolates respectively. However, the lowest was identified in urine sample (2.4%) as shown in the table 2.

Table 2 Distribution of A.	baumannii isolates	According to the So	ource of clinical specimens

Source of isolate	A. baumannii isolates No (%)	P value
Burn	15(36.5)	0.00004

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Surgical wound	14(34.1)	
Sputum	6(14.6%)	
CSF	3(7.3%)	
Blood	2(4.8%)	
Urine	1(2.4%)	
Total of samples	41(100)	

3.3. Comparison of A. baumannii isolates based on different age groups and gender

The results in the table below showed that the burns and surgical wounds were the most collected samples under the age (41-60) years which contain *A.baumannii* bacteria, so it could be picked up from the total study count (41) bacteria. While the minimum numbers of *A. baumannii* were isolated from C.S.F., sputum, blood, and urine under different age groups. Statistically these differences were non-significant (P-vale = 0.24) as shown in the table 3.

In terms of Gender, it can be demonstrated that *A. baumannii* was mostly isolated from male group compared with female group from different clinical samples, 22 bacteria versus 19 from total study count (41) cases. Statistically this difference was non- significant (P-vale = 0.67) as explained in the table 4.

Table 3 Distribution the number and percentage of A. baumannii according to the clinical samples with age groups

Categor	rical age	Number and percentage of A.baumannii / source of clinical samples						Total No.	P-value
group		Burn	Wound	Sputum	C.S.F	Blood	Urine	(all cases)	r-value
<1	N (%)	0	0	0	1	0	0	1	
years	IN (70)	(0.0%)	(0.0%)	(0.0%)	(33.3%)	(0.0%)	(0.0%)	(100.0%)	
(1-20)	(1-20) years N (%)	3	4	1	1	0	0	9	
years		(20.0%)	(28.6%)	(16.7%)	(33.3%)	(0.0%)	(0.0%)	(100.0%)	
(21-40)	NI (0/)	4	4	2	1	1	1	13	
years	N (%)	(26.7%)	(28.6%)	(33.3%)	(33.3%)	(50.0%)	(100.0%)	(100.0%)	*0.24
(41-60)	NI (0/)	8	6	2	0	1	0	17	(N.S)
years	N (%)	(53.3%)	(42.9%)	(33.3%)	(0.0%)	(50.0%)	(0.0%)	(100.0%)	
> 60	NI (0/)	0	0	1	0	0	0	1	
> 00	N (%)	(0.0%)	(0.0%)	(16.7%)	(0.0%)	(0.0%)	(0.0%)	(100.0%)	
Total	NI (0/)	15	14	6	3	2	1	41	
Total	N (%)	(100.0%)	(100.0%)	(100.0%)	(100.0%)	(100.0%)	(100.0%)	(100.0%)	

* Chi-square, N.S; non-significant

Table 4 The number and	percentage of A. l	paumannii according to th	e clinical samples with gender

Condon		Number and percentage of A. baumannii / source of clinical samples Total No.						D 1	
Genu	Gender		Wound	Sputum	C.S.F	Blood	Urine	(All cases)	P –value
Males	N (%)	8 (53.3%)	7 (50.0%)	3 (50.0%)	1 (33.3%)	2 (100.0%)	1 (100.0%)	22	
Females	N (%)	7 (46.7%)	7 (50.0%)	3 (50.0%)	2 (66.7%)	0 (0.0%)	0 (0.0%)	19	*0.67 (N.S)
Total	N (%)	15 (100.0%)	14 (100.0%)	6 (100.0%)	3 (7.1%)	2 (100.0%)	1 (2.4%)	41	

* Chi-square, N.S; non-significant

Furthermore, it can be obviously noticed from the table 3 and 4 above that most *A. baumannii* isolates were isolated from burn and surgical wounds under the (1-20), (21-40), and (41-60) age groups with 26.8 %, 34.1 %, and 36.5 % respectively. While the *A. baumannii* infection was rarely reported in infants who are less than one year and old age groups more than 60 years.

3.4. Isolation and identification of A. baumannii isolates

3.4.1. Culture and identification

Overall, All the *A. baumannii* isolates were phenotypically identified on both Blood and MacConkey agar with some different characteristics observed. The morphology of colonies has been seen on the blood agar within 24 h at 37°C. The color growth was white to gray and tiny circular shapes with no hemolysis noticed on colonies and surrounding area due to having no enzyme to hemolyze blood. While, the colonies properties which were appeared on the MacConkey agar at the same lab conditions can be discriminated with mucoid, faint pink, non-fermented lactose sugar, rounded and tiny shapes.

Also, a specific and rapid isolation of *A. baumannii* was conducted based on CHROMagar (HiCrome TM) with adding Multidrug resistance selective supplement (FD271). All isolates of growth including (MDR) appeared within (24 to 48 h) at 37°C, as light purple color with halos rounded the colonies. The positive control of *A. baumannii* (ATCC, 19606) was cultured and the results compared with all isolates to give 100% consistency.

3.4.2. Biochemical Testing of A. baumannii

Biochemical tests have been manually accomplished on the bacterial isolates from different clinical sources to show the results listed in the table 1.

3.5. Results of API20 E

API 20E system was used to accurately identify *A. baumannii* and obtain more sensitive identification results than other methods. The results were identical with the specific reference number of *A. baumannii* in API20E index. API 20 E was compared with conventional tests to reveal 92% accuracy [11].

3.6. Identification of A. baumannii used by VITEK® 2 system

In the current study, 41 isolates were tested by VITEK to be identified as *A. baumannii* using identification card of gram-negative bacteria (BioMérieux) by showing an interesting percentage of accuracy (99%) [11][13].

3.7. Antibiotics susceptibility profile

It was noticed that the vast majority of *A. baumannii* showed a high level of resistance to most of antibiotics such as Piperacillin (97.5%), Piperacillin/ Tazobactam, Ceftazidime (87.8%), Meropenem (85.3%), Tri/slphamethoxazole (82.9%), Levofloxacin (80.4%), Imipenem, Ciprofloxacin (78%), Gentamycin (75.6%), Amikacin (73.1%), Netilmicin (68.2%) and Tobramycin (60.9%). However, the isolates produced an extreme low resistance to some antibiotics particularly Colistin (0%), Tigecycline (2.4%) and Tetracycline (31.7%) as mentioned in the table 5

Table 5 Distribution of resistant, sensitive and intermediate isolates obtaining from Antibiotics susceptibility test

Antibiotics	Susceptibility Test	No	%	χ^2	P-value
Meropenem	R	35	85.3	-	
	Ι	1	2.4	48.143	0,0001
	S	6	14.6	46.145	
	R	32	78		
Imipenem	Ι	1	2.4	38,683	0,0001
	S	8	19.5	58,085	
	R	36	87.8		
Ceftazidime	Ι	0	0	21,429	0.0001
	S	6	14.6		
Dimenseillin /	R	36	87.8		
Piperacillin / Tazobactam	Ι	4	9.7	55.073	0.0001
Tazobactani	S	1	2.4		
	R	40	97.5		
Piperacillin	Ι	1	2.4	37.098	0.0001
	S	0	0		
	R	1	2.4		
Tigecycline	Ι	12	29.2	26.976	0.0001
	S	28	68.2		
	R	33	80.4		
Levofloxacin	Ι	0	0	15.244	0.0001
	S	8	19.5		
	R	32	78		
Ciprofloxacin	Ι	6	14.6	37.220	0.0001
	S	3	7.3		
	R	31	75.6		
Gentamycin	Ι	4	9.7	35.150	0.0001
	S	5	12.1		
	R	25	60.9		
Tobramycin	Ι	2	4.8	19.366	0.0001
	S	14	34.1		
Netilmicin	R	28	68.2		
	Ι	4	9.7	24.800	0.0001
	S	8	19.5		
T-4	R	13	31.7	5 200	0.074
Tetracycline	Ι	21	51.2	5.209	0.074

^{5.}

	S	9	21.9		
	R	0	0		
Colistin	Ι	1	2.4	37.098	0.0001
	S	40	97.5		
Tri/sulfamthoxazo	R	34	82.9		
le	Ι	0	0	17.780	0.0001
	S	7	17		
	R	30	73.1		
Amikacin	Ι	3	7.3	30.195	0.0001
	S	8	19.5		
Total	15	41	100%		
	R: Resistance	, I: Intermediate	, S: Sensitive		

All A. baumannii isolates (41) were classified into three groups: MDR, XDR, and PDR, based on antibiotic resistance by using AST. The percent of these classes were calculated to be 40.48%, 52.38%, and 0% respectively, compared with non-MDR that produced 7.14% of A. baumannii isolates, as explained in fig. 1.

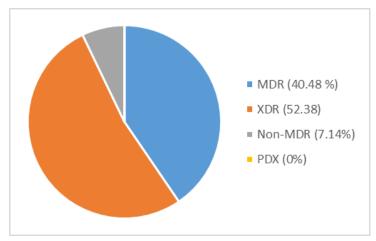


Fig. 1 Classes of antibiotic resistance pattern isolate of A. baumannii, Multi drug resistance (MDR), Extensive drug resistance (XDR) and Pan Drug resistance (PDR)

4. Discussion

One of the most infective bacteria, which transmit rapidly in hospitals, is A. baumannii. It could be explained by the long survival on the different surfaces as well as high resistance to most antibiotics, which recently led to a significant increase of infection. Consequently, it is important to find out a rapid method for identification of A. baumannii [7]. This will support the infection control measurements leading to increase the choices of antibiotics usage for infected patients' treatment [14][2]. Some conventional techniques such as routine culture and biochemical tests which are considered as a standard for cultivation methods were employed for early diagnosis to identify and detect of A. baumannii infections from different clinical sources [5]. The present findings also confirmed the study which was achieved in Iraq in 2016 [7] which reported that the highest percentage of identified A. baumannii in burn and wound. There are two reasons to interpret the presence of A, baumannii infection in burns and wounds more than other clinical sources. The burns and wounds are more vulnerable to contamination with the hospital environment, which helps to spread the resistant strains. Furthermore, most antibiotics have no ability to deeply penetrate the sites of burns and wounds [15]. Another interesting result that needs to be discussed, which is that no significant differences were found in gender and age groups of A. baumannii infection as observed in tables 3 and 4. However, it was clearly noticed that infants aged less than a year and older people aged more than 60 years are rarely infected with A. baumannii. The reason may belong to that those age groups have low mobility and are not exposed to more critical accidents such as burns and wounds compared with other age groups. The phenotypic identification shows various features when A. baumannii isolates were cultured on both Blood and MacConkey agar. More selective media was employed to identify A.baumannii, is CHROMagar, which contains a Multidrug resistance supplement (FD271). This media will help to completely confirm A. baumannii identification [15] [4]. Interestingly, the ability of A. baumannii to grow on the enrichment media at 44°C was obviously observed compared with other Acinetobacter species, which showed no positive growth. Conventional biochemical testing is still not efficient to identify A. baumannii in the microbiological domain [10]. Consequently, another technique system (API 20 E) has been employed to confirm A. baumannii from other species and obtain results that are more accurate.

It appeared that the majority of A. baumannii showed high resistance to most of antimicrobial agents such as Piperacillin (97.5%), Piperacillin/ Tazobactam, Ceftazidime (87.8%), Meropenem (85.3%), Tri/slphamethoxazole (82.9%), Levofloxacin (80.4%), Imipenem, Ciprofloxacin (78%), Gentamycin (75.6%), Amikacin (73.1%), Netilmicin (68.2%) and Tobramycin (60.9%) and Tetracycline (31.7%). Another study, identified that A. baumannii isolates were highly resistant to Imipenem and Meropenem (58.26%), Moreover, the author found that the highest resistance was to most antibiotics used especially Imipenem (100%) and Meropenem (90%) [16]. Also, it has been shown that clinical isolates of A.baumannii were highly resistant to aztreonam, Ceftraixone, Ceftazidime and Meropenem. However, the isolates produced an extreme low resistance to some antibiotics particularly Colistin (0%), and Tigecycline (2.4%) [17].

These interesting results can be interpreted that Colistin binds to lipopolysaccharides (LPS) of the outer membrane of G-ve bacteria leading to some changes in the bilayers of phospholipid composition. This phenomenon causes cell death by installing an osmotic imbalance [4]. A. baumannii isolates revealed resistance to most of the antibiotics with various percent; however, two antibiotics (Colistin and Tigecycline) were the most effective drugs to control the bacterium infection.

These results show a good compatibility with Babapour's study which was conducted in Iran [18]. A. baumannii resistances appeared with three classes Multi drug resistance (MDR), Extensive drug resistance (XDR) and Pan Drug resistance (PDR). Of the total A. baumannii (41) isolates identified in this study, the percentage of (MDR) including ATCC and (XRD) isolates, was calculated and showed 40.48% and 52.38% compared with non-MDR 7.14 % and PDR as mentioned in figure (1). The obtained results were compared with the study conducted in Iraq/Erbil in 2020; it was observed that XDR agreed with that result to show the highest percentage of isolates were XDR (51.35%). On the contrary, Non-MDR and MDR disagreed with the current study to be ranked in the second and third orders with (29.73%) and (18.92%) respectively [4].

5. Conclusion

A. baumannii is one of the most critical reasons that lead to nosocomial infection particularly in ICU wards. Some Identification methods were employed to isolate and investigate this bacterium. This study found that automated Vitek systems have identified A. baumannii with high efficiency comparing with other methods. The second interesting point was that A. baumannii showed a high percentage of resistance against many antibiotics and even to three classes in the same family generations resulted by AST. However, A. baumannii isolates produced a high sensitivity of two antimicrobial agents which are Colistin and Tigecycline with 0 % and 2.4 % respectively, therefore; those promising antibiotics were considered a good choice to treat MDR A. baumannii.

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