

IDENTIFICATION OF MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII*, ISOLATED FROM HOSPITALIZED PATIENTS IN DUHOK CITY

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ABSTRACT

Acinetobacter baumannii regard one of the most important nosocomial bacterium that may infect hospitalized patients especially in ICU, and can cause severe infections

The sensitivity pattern of *A. baumannii* revealed that the organism was highly sensitive to Colistin (93%), followed by Tigecycline (82 %). A high resistance rate (100%) was reported for Ceftriaxone, Cefixime, Ceftazidime, and Cefoxitin, while for Cefuroxime (92%) and for both Cefepime and Cefotaxime (98 %), also low sensitivity rate was shown by both Imipenem and Meropenem antibiotics against this bacteria with a sensitivity rate (17%), and due to resistotyping, a total of 9 different resistotype patterns were obtained; the most common resistotype was number (3) which include the following antibiotics : (PRL/TAZ, CAZ, CFP, AZT, MEM, IMP, AK, CN, NET, CIP, AMC, SXT, CRO, CFUR, LEV).

Finally, this study demonstrated the significance of *Acinetobacter baumannii* as a nosocomial bacterium that may infect hospitalized patients and cause severe illnesses, such as pneumonia, wound infections, bloodstream infections, meningitis, and urinary tract infections.

KEY WORDS: *A. baumannii*, Multidrug-resistance and Resistotyping patterns

INTRODUCTION

Acinetobacter baumannii bacterium is one of the most opportunistic pathogens which can cause severe nosocomial infections. It is one of the most problematic diseases for medical facilities (Fournier PE, 2006). The bacteria are Gram-negative coccobacilli or pleomorphic, aerobic, and non-motile (Stephen H., 2006). It was considered an important cause of opportunistic infections. Still, it is found to be non-pathogenic in healthy individuals (Al-Tamimi, 2022).

A. baumannii is one of the six most significant multi-drug resistant (MDR) bacteria found in hospitals worldwide. It has emerged as an important nosocomial pathogen associated with various infections. (Goudarzi H., 2019). The organism is typically resistant to various antimicrobial drugs, making its treatment even more difficult and challenging. Strains of these bacteria are resistant to all known antibiotics which have been identified now (Goudarzi H., 2019). This organism's success is attributable to both its capacity for long-term survival in a hospital setting and its quick development of antibiotic resistance (Fournier PE, 2006).

Because of its wide range of antibiotic resistance, it can be argued that this bacteria has been classified as a "red alert" human pathogen, alarming the medical community (Sebeny PJ, 2008). Even the prevalence of *A. baumannii* resistance isolates varies from one community to another. However, it has been rising over time (Garnacho-Montero, 2010), and this leads to limited therapeutic options, which contribute to increased morbidity and mortality (Hussein, N.H. 2013; Fishbain J, 2010; and Gordon NC, 2010).

A.baumannii has the ability to persist in the environment for prolonged periods. Poor and defective infection control practices increase the chances of cross-transmission between hospital patients. (Barman H, 2021). Risk factors for colonization or infection with multidrug-resistant *A. baumannii* include: (Ibrahim ME.2019).

- Prolonged length of hospital stay, exposure to an intensive care unit (ICU).
- Patients on mechanical ventilation.
- Prolonged exposure to antimicrobial agents.
- Recent surgical and invasive procedures, and
- Underlying severe illnesses.

A. baumannii infections have significantly increased worldwide, especially among hospitalized patients. (Garnacho, J., 2003), this

bacteria is more common in immunocompromised people, especially those who have had prolonged hospital stays or in intensive care units and who need mechanical ventilation, as well as patients who have wounds or other injuries (trauma patients) (Hussein, N.H., 2013; and Peleg A, 2008). When medical equipment such as ventilators, endotracheal incubators, and catheters are used, the risk of *A. baumannii* infections rises noticeably (Silago, V, 2022). Generally, the illnesses linked to this bacterium include pneumonia, wound infections, bloodstream infections, skin and soft tissue infections, urinary tract infections, meningitis, and peritonitis (Silago, V, 2022). *A. baumannii* -related infections can have death rates of up to (35%), and recently there have been increases in the prevalence of *A. baumannii* infections in some areas and places in Iraq (Al-Tamimi, 2022; and Ibrahim ME., 2019).

Due to their widespread occurrence in soil, water, and arid environments, *Acinetobacter* species are widely distributed in nature (Turton JF, 2006). And due to their long-term survival propensity on inanimate objects, they are frequently spread in the hospital environment. They are linked to inpatient and hospital staff skin colonization (Sebeny PJ, 2008; and La Scola B, 2004). *A. baumannii* specifically targets moist tissues as a pathogen, such as mucous membranes or skin regions that are damaged or exposed as a result of an accident (Nazer, L.H. 2015). And this bacteria are known to have been responsive to the majority of antibiotics in the 1970s, but it now appears to have considerable resistance to most first-line medications (Al-Tamimi, 2022 Ibrahim ME., 2019; Hussein N.H., 2013; and Fournier PE, 2006).

Finally, this study was performed to have updated information regarding *A. baumannii* infections and antibiotic susceptibility patterns in the current area. Thus, this study aimed to demonstrate the rate of *Acinetobacter baumannii* in hospitalized patients especially in ICU, among different clinical samples such as pneumonia, wounds, bloodstream infections, meningitis, and urinary tract infections. And also to evaluate the rate of multi-drug resistant *A. baumannii* strains and the resistotyping patterns of this bacterium by studying several isolates collected over a period

of time from clinical samples which settings in Duhok city.

METHODS

Acinetobacter baumannii Isolates Collection

Bacterial isolates were collected from October 2021 to the end of June 2022 at VIN and Duhok emergency hospitals. For getting the isolates, various specimens were taken from hospitalized adult patients aged between 18-75 years, and the majority of them were in the ICU department. The specimens included wounds, blood, CSF, sputum, and urine.

Bacterial Identification

Specimens were plated on culture media, including MacConkey agar and blood agar, followed by Gram stain. The identified isolates were further analyzed by using the VITEK 2 Compact System with a Gram-negative detection kit (GN-69 BioMérieux, France), which employs a fluorogenic methodology for organism identification.

Antibiotic susceptibility testing

The antibiotic susceptibility pattern was performed using the VITEK 2 system with a Gram-negative susceptibility card (AST – N 326 BioMérieux, France), which depend on the turbidimetric method for determine the susceptibility of each antibiotic against the bacteria.

RESULTS

A. baumannii isolates identification

A total of 72 *A. baumannii* isolates were identified from October 2021 to the end of June 2022. Identification confidence was good or excellent for 97% of the reported isolates. Isolate distribution indicated all isolates were obtained from adults aged 18–75 years, and all of the isolates were obtained from inpatients, mostly in the ICU department. Strains of this bacterium that were isolated include: 34 were isolated from sputum, 21 were isolated from wounds, 13 strains were isolated from blood cultures, 3 were isolated from cerebrospinal fluid, and 1 from urine as explained in Table number 1.

Table (1): Number and the rate of *A. baumannii* isolates from Specimens

Specimen source	Numb of <i>A. baumannii. isolates</i>	Percentages of isolates (%)
Sputum	34	47
Wound	21	29
Blood	13	18
CSF	3	4
Urine	1	2
Total	72	100 %

Antibiotics resistance patterns

The antibiotic resistance rate is explained in Table 2. Generally, as it showed in this table, the total resistance average of *A. baumannii* isolates to the antibiotics was (85%) while the total sensitivity rate was (14.5%). The resistance rate to the aminoglycoside antibiotic group ranged from (81%) for gentamicin to (90%) for Amikacin. At the same time, the resistance rate was (89%) for Imipenem and (83%) for Meropenem. While among the tetracycline group, the resistance rate was relatively low for Tigecycline at (18 %) whereas it was very high for Tetracycline (98 %). Also, from the result, several types of antibiotics were not or had very low effectiveness against *A. baumannii* isolates, this included Ampicillin with a resistance rate

(100%) and the cephalosporin group, which had a very high resistance rate of (100 %) which was reported for ceftriaxone, cefixime, ceftazidime, and cefoxitin, while for cefuroxime (92 %) and for both cefepime and cefotaxime (98 %). In addition, there was a very high resistance rate detected for Aztreonam antibiotics (98 %), and due to the Fluoroquinolones group were mostly ineffective. The resistance rate for ciprofloxacin and levofloxacin were (92%), and (89%) respectively.

The Trimethoprim/sulfamethoxazole had a resistance rate about (78%), while piperacillin/tazobactam had a (92%) resistance rate. And finally, the resistance rate for Colistin was very low at (7 %) which is regarded as the lowest resistance rate among the antibiotics

Table (2): Antibiotic Susceptibility Pattern for *A. baumannii* Isolates

AST for 72 isolates of <i>A. baumannii</i>					
	ANTIBIOTICS	Symbol	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)
1	Ampicillin	AMP	0 (0)	0 (0)	72 (100)
2	Co-Trimethoprim	SXT	16 (22)	0	56 (78)
3	Pipracillin-Tazobactam	PRL/TAZ	6 (8)	0 (0)	66 (92)
4	Cefuroxime	CFUR	0 (0)	0 (0)	72 (100)
5	Cefataxime	CTX	1 (2)	0 (0)	71 (98)
6	Cefoxitin	CFN	0 (0)	0 (0)	72 (100)
7	Cefixime	CFM	0 (0)	0 (0)	72 (100)
8	Ceftazidime	CAZ	5 (7)	1 (2)	66 (91)
9	Ceftriaxon	CRO	0 (0)	0 (0)	72 (100)
10	Cefepem	CPM	1 (2)	0 (0)	71 (98)
11	Imipenem	IMP	7 (9)	1(2)	64 (89)
12	Meropenem	MEP	11 (15)	1(2)	60 (83)
13	Amikacin	AK	4 (5)	0	68 (95)
14	Gentamycin	GN	14 (19)	0	58 (81)
15	Ciprofloxacin	CIP	6 (8)	0	66 (92)
16	Tigecycline	TIG	59 (82)	6 (8)	13 (18)
17	Colistin	COL	67 (93)	0	5 (7)
18	Levofloxacin	LEV	7 (9)	1(2)	64 (89)
19	Tetracycline	TE	4 (6)	0 (0)	68 (94)
20	Aztreonam	ATM	0 (2)	0 (0)	71 (98)
	Total Rate		14.5 %	0.5 %	85%

Resistotyping patterns

Table number (3) shows the resistotyping patterns of the isolates to commonly used antibiotics. It was found that all of the isolates were multiple resistant, meaning that all of them

were resistant to many types of antibiotics. The obtained results of resistotyping patterns (resistant profiles) showed that (72) isolates were multiple drugs resistant to antibiotics, and nine different patterns were found.

Table (3): Resistotyping patterns of *A. baumannii* isolates

Resistotyping patterns	Resistance Spectrum Phenotype	No. of isolates (%)
Resistotype 1	PRL/TAZ, CAZ, CFP, AZT, MEM, IMP, AK, CN, NET, CIP, AMC, CRO, CFUR, , LEV	14 (19)
Resistotype 2	CAZ, CFP, AZT, MEM, IMP, AK, NET,, AMC, CRO, CFUR,	6 (8)
Resistotype 3	PRL/TAZ, CAZ, CFP, AZT, MEM, IMP, AK, CN, NET, CIP, AMC, SXT, CRO, CFUR, , LEV	24 (33)
Resistotype 4	PRL/TAZ, CAZ, CFP, AZT, AK, CN, NET, CIP, AMC, SXT, CRO, CFUR,	2 (3)
Resistotype 5	PRL/TAZ, CAZ, CFP, AZT, AK, , NET, CIP, AMC, SXT, CRO, CFUR, LEV	2 (3)
Resistotype 6	PRL/TAZ, CAZ, CFP, AZT, MEM, IMP, AK,, NET, CIP, AMC, SXT, CRO, CFUR, LEV	2 (3)
Resistotype 7	PRL/TAZ, CFP, NET, CIP, AMC, SXT, CRO, CFUR, LEV	2 (3)
Resistotype 8	PRL/TAZ, CAZ, CFP, AZT, MEM, IMP, AK, CN, NET, CIP, AMC, SXT, CRO, CFUR, Tig, , LEV	12 (17)
Resistotype 9 (11)	PRL/TAZ, COL, CAZ, CFP, AZT, MEM, IMP, AK, CN, NET, CIP, AMC, SXT, CRO, CFUR, Tig, , LEV	8
Total		72 (100)

DISCUSSION

This study showed that *Acinetobacter baumannii* is an important nosocomial bacterium that cause infections in hospitalized patients, such as pneumonia, wound infections, bloodstream infections, meningitis, and urinary tract infections. The current study result is similar to other studies that cited that this bacterium has emerged as one of the most troublesome pathogens in health care centers (Peleg A.Y., 2008). Also, the study confirmed that infections caused by this bacterium are typically more prevalent in immunocompromised or patients in intensive care units (ICU). And this bacterium has become a common cause of different hospital infections (Chen L.K., 20017; Peleg A.Y., 2008; and Fournier PE, 2006).

The occurrence and spread of this bacterium in the Duhok area may be due to recent problems and conflicts in Iraq and the Middle East. A large number of immigrants and refugees, a lack of appropriate medical care, and the coronavirus pandemic caused increased numbers of patients with serious diseases, many of whom require ICU admission and ventilation. Therefore all these factors increase the opportunity to get nosocomial infections, including *A. baumannii*, and raise the incidence rates of this bacterium (C.M., 2018; Harding C.M., 2017 and Choi J.Y.2005). Therefore, continued monitoring of this type of bacteria is critical to limit the spread of this pathogen.

This study also showed that most isolates were from hospitalized patients and were isolated mainly from sputum, followed by infected wounds and then by a blood infection, especially with patients in ICU and surgical wards. The findings were similar to those of a Jordanian study that showed an increased incidence of *A. baumannii* infections in hospitalized patients, especially in the ICU (Al-Tamimi, 2022 and Ibrahim ME., 2019).

Antibiotic susceptibility patterns in this study showed high resistance to most antibiotics which include groups of cephalosporins, carbapenems, fluoroquinolones, aminoglycoside and ampicillin, while a moderate resistance for trimethoprim/sulfamethoxazole was observed. The lowest resistance rates was for Colistin and Tigecyclines. This study found that the total rate of MDR isolates of *A. baumannii* was 85 %, while the total rate of sensitivity was only 14.5%, and this agrees with many others studies which found high resistance of *A. baumannii* isolated to a wide

spectrum of antibiotics (Al- Tamimi, 2022; Jale M, 2015; Hussein, N.H. 2013 and AL-Saleem, N. H. 2013). The study done by (Hussein, N.H 2013) has found a high rate of MDR *A. baumannii* against the antibiotics ranging 88% which was near to the present result. Also, others in Jordan and IRAN had reported MDR rates of 71 % and 77.6% respectively (Bahador A.;2015 and Nermen, D. 2009).

In previous studies, Imipenem and Meropenem antibiotics were considered the most effective treatments against beta-lactamases and infections caused by multidrug-resistant *A. baumannii* (Luyt C.E., 2014). But the current study identified that *A. baumannii* isolates were highly resistant to this type of antibiotic (89 %), while other studies (Al-Mash'hadani, H.I.J. 2010) reported low resistance to Imipenem, which doesn't agree with the current study. Others found moderate resistance to Imipenem and Meropenem 58.26% (Hussein, N.H., 2013). The increasing numbers of resistant isolates to this group of antibiotics indicates a dramatic reduction in the existing therapeutic options (Sheng W.-H., 2010).

The results of antibiotic sensitivity test in the current study showed low resistance rate to the Colistin and Tigecycline. The study found that the most active drugs against *A. baumannii* were Colistin (91%) and Tigecycline (89%). This result is similar to an Egyptian study (Al-Agamy, M.H.; 2014), which found that (95.9%) of *A. baumannii* isolates were sensitive to Colistin. In addition, a Turkish study mentioned that the most common active drugs against *A. baumannii* were Tigecycline and Colistin (Bayram, Y.;2013). Tigecycline antibiotic is a new glycolcycline agent showing bacteriostatic activity against MDR-Acinetobacter species (Sunenshine R.H., 2007). In some other studies, Acinetobacter isolates were found to be less susceptible to Tigecycline (Velkov T., 2013and Choi J.Y.2005), but even so, it is still an effective antibiotic against MDR *A. baumannii* isolates with the Colistin antibiotic. Colistin is increasingly being used as the last-line therapeutic option against MDR Gram-negative bacteria as a whole, such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and also *Acinetobacter baumannii* (Velkov T., 2013). This antimicrobial drug have various mechanisms to kill bacteria, and the most important one that its ability to induce the lysis of bacterial membranes (Yu Z., 2015). Although the use of Colistin antibiotic has limitations due to its toxicity, it is often used for treating life-threatening infections (Gounden, R. 2009). It

should be noted that colistin-resistant *A. baumannii* strains have been reported in various regions (Gounden, R. 2009), but the rate was low in the current area.

This study also reported very high resistance to the most common antibiotics, especially the Cephalosporin group, which includes Cephalexin, Cefixime, Cefotaxime, ceftriaxone, and ceftazidime, which mostly the rate of resistance of these antibiotics reached (100%). The high resistance of this bacterium to the Cephalosporins has been reported in other studies (Tian G.-B., 2011). When they mention the strains of *A. baumannii* can produce extended-spectrum class C-lactamases, which do confer resistance against Cephalosporins. The study done by (AL-Salem, N. H. 2013) showed that the clinical isolates of *A. baumannii* were highly resistant to Aztreonam, Penicillin's group, and Meropenem, as it mentioned in this study. Also, the study done by (Nermen, D. (2009) in Jordanian hospitals showed that the susceptibility of *A. baumannii* isolates indicated a high rate of resistance to most antibiotics, as it also mentioned in the current study. Another study found that all *A. baumannii* isolates had high resistance to Nitrofurantoin, Amoxicillin, Carbencillin, Ceftazidime, Cefepime, and Aztreonam, but low or no resistance to Carbapenems and Colistin. (Ramoul, A.2013).

The results in this study showed some sensitivity rates toward Gentamycin antibiotic with rate (22%) and Co-Trimethoprim with rate (19 %). The difference in antibiotic susceptibility patterns may happen in different geographical regions. This may depend on the recruited patients for the study, the types of antibiotics, and how many of them have been used in each area.

The high rate of MDR *A. baumannii* isolates reported in this study may be due to the fact that the population selected for sampling in this research is mainly composed of critically ill hospitalized patients with weak immunity, they are more likely to get MDR *A. baumannii*. Other studies also agree with these findings (Chuang Y.-C., 2011; Blossom D.B, 2003), indicating that critically ill patients are ten times more likely to have an MDR bacteria, including *A. baumannii*. That means the hospitalization duration is the important risk factor associated with *A. baumannii* MDR. The more time spent in the hospital, means the greater risk of exposure to the MDR bacteria, especially in the ICU, where patients mostly have weak immunity.

Resistotyping is a phenotypic method that tests bacterial strains against a set of arbitrarily chosen antibiotics. A resistance pattern characteristic of a strain is generated and is believed to describe the isolates for epidemiological purposes (Adesida SA., 2003). The current study's results revealed that the (72) isolates of *A. baumannii* belonged to (9) distinct resistotype patterns. The resistotype number (3) has a much higher frequency rate, comprising 33%, followed by Resistotype (1) with a rate of (14%) and Resistotype (8) with a rate of (12%). In general, this simple typing system provides discriminatory between strains and can determine relatedness among isolates of *A. baumannii* to trace the source of infections in the current environment. New and more effective antibiotics may be needed to treat the infections that may be caused by the different resistotypes isolates of this bacterium.

CONCLUSIONS

In conclusion, almost (85%) of *A. baumannii* isolates from this study were multi-drug resistant. Hospitalized patients being critically ill and having pneumonia or infected wounds were significantly associated with MDR isolates. These infections are associated with increased use of mechanical ventilation in ICUs and other critical units in the hospital. Because of high rates of antibiotic resistance which were observed in this study, treatment options are limited. The lowest resistance rates were observed for Colistin and Tigecycline making them the most effective treatment options.

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الخلاصة

هدفت هذه الدراسة إلى تحديد معدل مقاومة بكتيريا (*Acinetobacter baumannii*) ضد عدد من المضادات الحيوية وقد تم عزل هذه البكتيريا من عينات سريرية مختلفة من المرضى الراقدين في المستشفى وكذلك تم تحديد أنماط مقاومة العزلات للمضادات الحيوية .

خلال فترة تسعة أشهر من فبراير 2021 إلى يونيو 2022 تم أخذ عينات مختلفة للحصول على عزلات *Acinetobacter baumannii* من المرضى البالغين والراقدين في المستشفى ، الذين تتراوح أعمارهم بين (18-75) وكان معظمهم في قسم العناية المركزة ، والعينات المأخوذة شملت التهابات الجروح ، التهاب الدم ، التهاب السائل النخاعي ، التهابات الرئة ، والمجاري البولية و تم زرع جميع العينات على وسائط الاستزراع ، بما في ذلك أجار ماكونكي MacConkey ، وأجار الدم ، وتم تحديد أنماط الحساسية للمضادات الحيوية باستخدام جهاز Vitek 2 Compact.

أظهرت هذه الدراسة أن بكتيريا *Acinetobacter baumannii* تعتبر مهمة في عدوى المستشفيات مثل التهابات التنفسية والتهابات الجروح وعدوى التهاب الدم والتهاب السحايا والتهابات المسالك البولية. وقد أظهر نمط الحساسية لـ *Acinetobacter baumannii* أن البكتيريا كانت شديد الحساسية للمضاد الحيوي كوليستين Colistin (93%) يليه Tigecycline (82%) المضاد تيجيساكيلين ، و كان معدل المقاومة مرتفع جدا (100%) للمضادات Ceftriaxone و Cefixim و Cefazidime و Cefoxitin ، بينما بالنسبة للمضاد Cefuroxime كان (92%) ولكل من Cefepime و Cefotaxime كان (98%) ، وقد أظهرت كل من المضادات الحيوية Imipenem و Meropenem معدل حساسية منخفض ضد هذه البكتيريا (17%). وأخيرًا وبالنسبة لأنماط المقاومة فقد تم الحصول على (9) أنماط مقاومة مختلفة من هذه البكتيريا والنمط رقم 3 كان الأكثر مقاومة والذي تضمن المضادات التالية (PRL/TAZ, CAZ, CFP, AZT, MEM, IMP, AK, CN, NET, CIP, AMC, SXT, CRO, CFUR, LEV).

الكلمات المفتاحية: مقاومة الأدوية المتعددة ، أنماط المقاومة ، المضادات الحيوية