Antibiotic Resistance Pattern and Prevalence of Multi-Drug and Extensive Resistant *Acinetobacter Baumannii* Isolates from Clinical Specimens after Military Operations Western Iraq

Askeri Operasyonlar Sonrası Klinik Örneklerden Çok İlaca ve Kapsamlı Dirençli *Acinetobacter Baumannii* İzolatlarının Antibiyotik Direnç Modeli ve Prevalansı Batı Irak

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ABSTRACT

The main objective of this paper is to investigate the multi-drug resistance among *A. baumannii* isolates that are isolated from clinical specimens in Ramadi Teaching Hospital, after Military Operations western Iraq. The total number of patients who were culture positive was eighty-eight out of two hundred and thirteen (88%), during the period from April 2011 to June 2012. Thirty-one *A. baumannii* clinical isolates were selected. IPM-EDTA-disk synergy test was used for phenotypic expression of MBL producing *A. baumannii* and minimal inhibitory concentration (MIC) of antimicrobial susceptibility test by Vitek2 system for antibiotic resistance pattern and prevalence of multi-drug resistant *A. baumannii.* Twenty-eight out of thirty-one isolates (90.32%) of *A. baumannii* were determined MBL producers by using IPM-EDTA-disk synergy test (positive), while twenty-seven out of thirty-one isolates (87%) were resistance to Imipenem by MICs obtained by VITEK-2. Results reported that all isolates (31 isolates/100%) of *A. baumannii* were Multi-drug resistant (MDR), while 27 isolates (87%) were Extensively Drug-Resistant (XDR) and ten isolates (32.25%) were Pan Drug-Resistant (PDR). Antibiotic resistance pattern showed all isolates exhibited a high rate of resistance (100%) to Ampicillin, Cefazolin, Cefoxitin, Nitrofurantoin, and Trimethoprim/sulfamethoxazole. Most isolates (96.7%) were resistance to Piperacillin/Tazobactam. Resistance to anther antibiotics varied among isolates of *A. baumannii*, were (93.5%) for Ceftriaxone and Cefepime, (90.3%) for Ampicillin/Sulbactam, (87%) for Imipenem and meropenem, (80.6%) for Gentamicin, (71%) for Ceftazidime, (67.7%) for Amikacin and Ciprofloxacin and (64.5%) for Tobramycin. Furthermore, the lowest resistance was to Levofloxacin (9.6%), while 58% of isolates (18/31) were sensitive to Levofloxacin.

Keywords: Multi-drug resistant *Acinetobacter baumannii*, Metallo-beta-lactamase production, Carbapenem-resistant *Acinetobacter baumannii*

Received: 01.07.2020 **Accepted:** 05.26.2020

ÖZET

Bu makalenin temel amacı, Irak'ın batısındaki Askeri Operasyonlar sonrasında Ramadi Eğitim Hastanesi'nde klinik örneklerden izole edilen *A. baumannii* izolatları arasındaki çoklu ilaç direncini araştırmaktır. Nisan 2011-Haziran 2012 döneminde kültür pozitif olan toplam hasta sayısı iki yüz on üçten seksen sekizi (%88) idi. Otuz bir *A. baumannii* klinik izolatı seçildi. MBL üreten *A. baumannii*'nin fenotipik ekspresyonu için IPM-EDTA-disk sinerji testi ve antibiyotik direnç paterni ve çoklu ilaca dirençli *A. baumannii* prevalansı için Vitek2 sistemi tarafından antimikrobiyal duyarlılık testinin minimal inhibitör konsantrasyonu (MIC) kullanıldı. *A. baumannii*'nin otuz bir izolatından yirmi sekizi (%90.32) IPM-EDTA-disk sinerji testi (pozitif) kullanılarak MBL üreticisi olarak belirlenirken, otuz bir izolattan yirmi yedisi (%87) dirençli idi. VITEK-2 tarafından elde edilen MIC'ler ile imipenem'e. Sonuçlar, *A. baumannii* izolatlarının tamamının (31 izolat/%100) Çoklu ilaca dirençli (MDR), 27 izolatın (%87) Kapsamlı İlaca Dirençli (XDR) ve on izolatın (%32,25) Pan İlaç olduğunu bildirdi. -Dayanıklı (PDR). Antibiyotik direnç paterni, tüm izolatların Ampisilin, Sefazolin, Sefoksitin, Nitrofurantoin ve Trimetoprim/sülfametoksazole karşı yüksek oranda (%100) direnç sergilediğini gösterdi. İzolatların çoğu (%96.7) Piperasilin/Tazobaktam'a dirençliydi. Anter antibiyotiklere direnç *A. baumannii* izolatları arasında farklılık göstermekle birlikte, Ceftriaxone ve Cefepime için (%93.5), Ampisilin/Sulbaktam için (%90.3), İmipenem ve meropenem için (%87), Gentamisin için (%80.6), (%71) idi.) Seftazidim için, (%67.7) Amikasin ve Siprofloksasin için ve (%64.5) Tobramisin için. Ayrıca en düşük direnç Levofloksasine (%9.6) karşı iken, izolatların %58'i (18/31) Levofloksasine duyarlıydı.

Anahtar Sözcükler: Çoklu ilaca dirençli *Acinetobacter baumannii*, Metallo-beta-laktamaz üretimi, Karbapenem dirençli *Acinetobacter baumannii*

Geliş Tarihi: 07.01.2020 **Kabul Tarihi:** 26.05.2020

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doi:http://dx.doi.org/10.12996/gmj.2021.87

INTRODUCTION

Acinetobacter baumannii is a glucose-non-fermentative, Gram-negative coccobacillus that is found to be one of the main factors causing nosocomial infections connected to elevated morbidity and mortality (1, 2). It is capable of causing both community and hospital-acquired infections targeting critically sick patients with breaches in airways and skin integrity. Hospital-acquired infections are the principal characteristic of multi-drug resistant *A. baumannii* mainly causing surgical site infection, urinary tract infection, respiratory tract infection, and septicaemia(3). Recently, it has been considered as a "red alert" human pathogen. It generates alarm among the medical staff arising mostly from its extensive antibiotic resistance spectrum (4).

In conflict zones, *A. baumannii* is considered as the main cause for concern, and has obtained significant notoriety in the resent desert conflicts in Iraq, earning it the moniker "Iraqibacter''. Expressly, it was noted that the occurrence frequency of multidrug-resistant (MDR) bacteremia was high among members of US Army service following the Operation Iraqi Freedom (1). The antimicrobial resistance has recently been identified by the World Health Organization (WHO) as one of the three most essential issues human health is currently facing. Acronym "ESKAPE, standing for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp*." are identified as the most common and severe MDR pathogens. Based on the CDC (Centre for Disease Control), two-thirds of all hospital-acquired infections are caused by the six ESKAPE bacteria (4, 5).

Starting a decade ago, the terms "pan drug resistance", "extensively drug resistance," and "multidrug resistance" have been used commonly for *A. baumannii* strains to designate (i) resistance to all, (ii) but one or two, and (iii) to three or more classes of potentially active antimicrobial agents, respectively (6, 7). *A. baumannii* hospital strains are generally multidrug-resistant. The issue gets more complicated by increasing the resistance to broad-spectrum antibiotics including carbapenems, the drugs of choice for nosocomial *A. baumannii* infections (8). Carbapenems are considered the last-line drugs for dealing with infections caused by multiresistant Gram-negative bacilli (9). Meropenem or imipenem select respectively for carbapenem-resistant Gram-negative organisms, including pre-existing carbapenem-resistant *A. baumannii*.

Recently, with the growing use of carbapenems in clinical practice, the spread of carbapenem-resistant pathogens like *Acinetobacter baumannii* and *Pseudomonas aeruginosa* now poses a significant threat to human health (10, 11). This study aims to look into multi-drug resistance among *A. baumannii* strains isolated from clinical specimens during 2011-2012.

MATERIALS and METHODS

Collection of samples

Swabs were taken from different anatomical sites (e.g. bone, Joints, connective tissues) of Two Hundred and Thirteen patients with wound infections (osteomyelitis, burn infection. etc). The swabs were collected from Ramadi Teaching Hospital patients, including (in and out) patients and Burn Unit from April 2011 to June 2012. Collected swabs of different age groups (male and female) aged between <2 and 80 years. All isolates were bacteriologically identified using conventional and VITEK® 2 system according to criteria mentioned by bioMérieux (12). 188 of 213 patients were culture-positive (88.26%).

Thirty-one out of forty-one isolates (75.6%) of *Acinetobacter baumannii* were chosen to test MBL producers by IPM-EDTA combined disc then selected to study antimicrobial susceptibility test by Vitek2 system.

Confirmatory testing for Metallo beta-lactamase production (Imipenem-EDTA combined disc Synergy)

Imipenem-EDTA combined discs were made by adding EDTA solution to 10µg imipenem discs to obtain a concentration of 750 µg. The discs were dried immediately in an incubator and stored at 4°C in an airtight vial. Bacterial isolates were adjusted according to McFarland 0.5 turbidity standard and were inoculated to Mueller Hinton agar. A 10-µg-imipenem disc and imipenem plus 750 µg EDTA were placed on Mueller Hinton agar, then overnight incubation, the established zone diameter difference of ≥ 7 mm between imipenem disc and imipenem plus EDTA was interpreted as EDTA synergy positive (13).

Statistical analysis

Statistical significance was taken with the threshold P-value < 0.05. The significant differences were detected by using the goodness fit test within non-parametric statics including chi-square (cross-tabulation) test; Chi-square test was used to study the correlation (dependence) between the type of tests (the type of antibiotics used by Vitek2 system). Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows (Version 22.0, SPSS Inc., Chicago, IL, USA).

The study was approved by Local Ethics committee in University Of Anbar. Informed consent/assent was obtained from the patients with burns (213 males and females) in Teaching Ramadi Hospital in Ramadi.

RESULTS and DISCUSSION

The target of this study was *Acinetobacter baumannii*. The percentage isolation of *Acinetobacter baumannii* from Burn Unit were the highest at 90.34% (28/31 isolates) versus 9.67% (3/31isolates) from the wound (Fig. 1). This may be due to the prevalence of low-level socioeconomic groups of patients in whom adverse hygienic conditions prevail; malnutrition might also participate in the earlier formation of infection. Family members and hospital staff predominantly often touch hospitalized patients. These contact activities can quickly spread out the infection. Also, insufficient measures to stop cross-infection between visitors and burn unit workers can affect. In burn units, the most important causes for nosocomial infections are compliance deficiency with hygiene rules, inadequate sterilization of fomites and inappropriate antibiotic use (14). Generally, *Acinetobacter baumannii* infection patients have more severe burns, more co-morbidities, and can stay longer than colonization patients (15).

The current study also shows that the percentage of female infected with *Acinetobacter baumannii* is higher than the male; 64.51% (20/31 isolates) versus 35.48% (11/31) for male. All of the female isolates are from Burn Unit 100% (20/20 isolates) versus 72.27% (8/11 isolates) for male (Fig. 1). This result agrees with the study which is performed by Wisplinghoff *et al.* (16). In their study, the researchers have also confirmed that female gender was a separate dangerous factor for nosocomial *A.baumannii* bacteraemia in burn patients. The researchers did not find in their study interpretation of the rule of female gender but in the current study, the reason may be attributed to the nature of Eastern women's work in the kitchens and proximity to sources of burning (Heater, Ovens & Cookers). The majority of women in the present study were burned to the above cause. This result was in agreement with similar studies performed in Iran, in which females were the victims of burns more frequently than males (17).

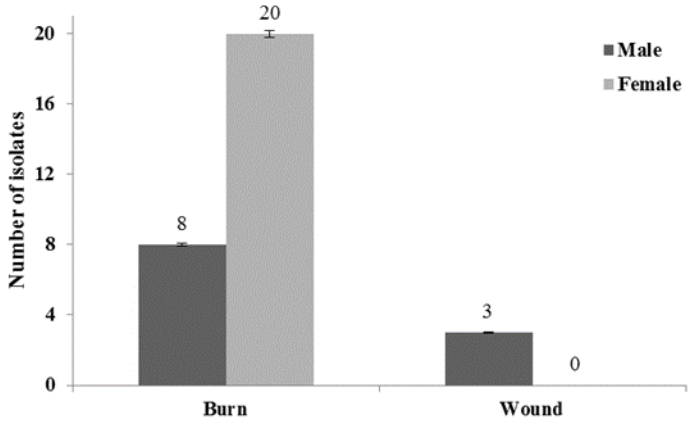

Figure 1 No. of *Acinetobacter baumannii* isolates as to the sex and type of specimen

To detect MBL-producing isolates, many phenotypic methods are available. However, the Clinical and Laboratory Standards Institute (CLSI) does not recommend any "standardized protocol for screening of MBLs". The method using a disc with Imipenem plus 750 µg of EDTA (combined disc method) is simple to perform and highly sensitive in differentiating MBL-producing isolates (13).

Twenty-eight out of thirty-one isolates (90.32%) of *Acinetobacter baumannii* were MBL producers by IPM-EDTA-disc synergy test (positive) (Table 1) (Fig. 2 and 3). The determined zone diameter difference of ≥ 7 mm between imipenem disc and EDTA plus imipenem was interpreted as EDTA synergy positive (appearance of an enlarged zone of inhibition was interpreted as EDTA-synergy test positive) (18).

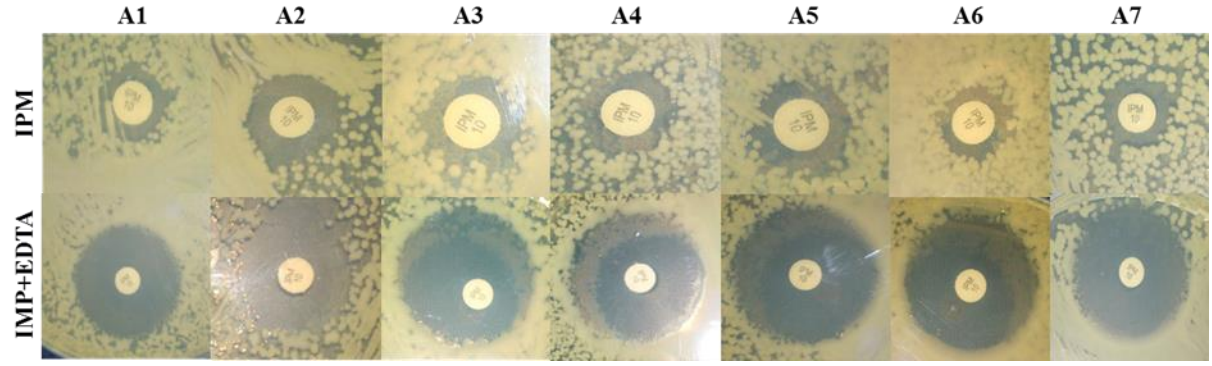

Figure 2 The positive result for Screening and Confirmatory Testing for metallo beta-lactamase production (mm) by *A. baumannii* on Muller-Hinton agar

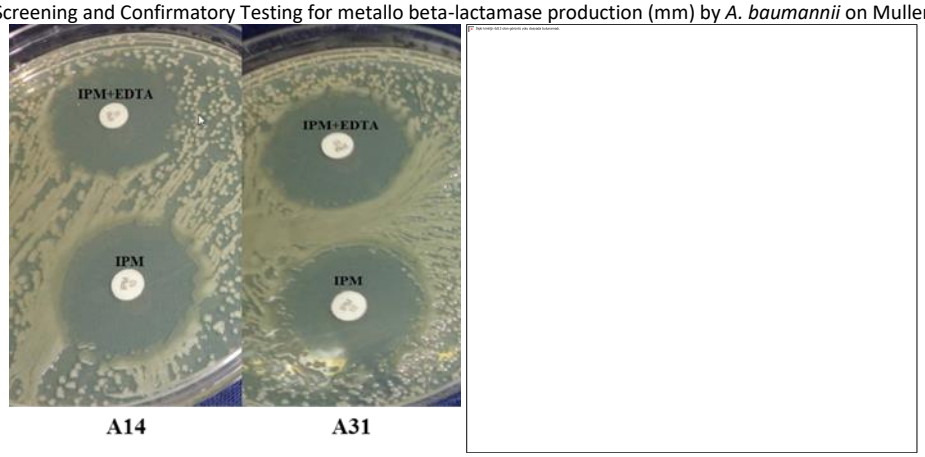

Figure 3 The negative result for Screening and Confirmatory Testing for metallo beta-lactamase production (mm) by *A. baumannii* on Muller-Hinton agar

Table 1 Testing for potential Metallo-β-lactamase producer and MIC of an Imipenem* by Vitek2 system for *A. baumannii* isolates

No.	No. of Isolates	Testing For metallo beta-lactamase production (mm)			*Result of* MIC *For* IPM by Vitek2 system
		IPM+EDTA	IPM	Result	
1	A1	25	10	R	R*
2	A2	20	12	R	R*
3	A3	25	9	R	R*
4	A4	27	9	R	S*
5	A5	19	11	R	R*
6	A6	26	10	R	R*
7	A7	26	6	R	R*
8	A8	29	10	R	R*
9	A9	26	11	R	R*
10	A10	20	12	R	R*
11	A11	20	12	R	R*
12	A12	21	11	R	R*
13	A13	25	11	R	R*
14	A14	26	26	S	S*
15	A15	27	27	S	S*
16	A16	26	9	R	R*
17	A17	26	9	R	R*
18	A18	25	10	R	R*
19	A19	26	9	R	R*
20	A20	24	10	R	R*
21	A21	24	10	R	R*
22	A22	25	9	R	R*
23	A23	23	10	R	R*
24	A24	24	10	R	R*
25	A25	24	10	R	R*
26	A26	23	10	R	R*
27	A27	25	10	R	R*
28	A28	24	11	R	R*
29	A29	23	9	R	R*
30	A30	25	10	R	R*
31	A31	26	25	S	S*
%				90.32	87.09

Twenty-seven out of thirty-one isolates (87%) of *Acinetobacter baumannii* were resistant to Imipenem by MICs obtained by VITEK-2 (MIC ≥16 µg/mL) (Table 1), while the other four isolates were sensitive to imipenem (MIC ≤4 µg/mL) (19). The results above of *Acinetobacter baumannii* displayed unusually high-level of imipenem resistance. The occurrence of imipenem-resistant *A. baumannii* was widely noticed in the Middle East. The results of the current study are in agreement with the many studies in the United Arab Emirates and Bahrain (20). The extensive use of carbapenems in the Middle East had generated a selective antibiotic pressure, which caused an increased spread of carbapenem-resistant *A. baumannii*.

MICs obtained by VITEK 2 in this study match with the results of confirmatory testing for Metallo beta-lactamase production in the same study (Table 1). The results of the current study are in agreement with the comparative study conducted by Kottahachchi *et al*., in 2012 at which the researchers compared the application of the E test and the VITEK 2 system in susceptibility testing of resistant strains of *A. baumannii* and *P. aeruginosa* to meropenem. They concluded that VITEK 2 is a valid technique to obtain MIC to meropenem for *A. baumannii* and *P. aeruginosa* (21). The technology of the VITEK 2 system enables fast diagnosis, within hours rather than the days required for classical methods (22).

Table 2. Result of minimal inhibitory concentration (MIC) of Antimicrobial Susceptibility Test for isolates of *Acinetobacter baumannii* by Vitek2 system (MDR, XDR & PDR *Acinetobacter*)

Development and prevalence of *Acinetobacter* species, resistant to most of the antimicrobial activity, is an area of great worry. The abbreviations such as MDR (Multi-drug resistant), XDR (Extensively Drug-Resistant) and PDR (Pan Drug Resistant) have been used in research publications with varied definitions, leading to confusion in the correlation of data from different researches. 'MDR isolates defined as the isolate resistant to at least one agent in three categories of antibiotics: all penicillins, cephalosporins (including inhibitor combinations), aminoglycosides and fluoroquinolones. 'XDR isolate that is resistant to at least one agent in the three categories of antibiotics described above (MDR) and also be resistant to carbapenems (23). Ultimately, 'PDR isolate was defined as non-susceptibility to all agents in all antibiotic classes (24).

Results of this research showed that all isolates (31 isolates) of *A. baumannii* (100%) were found resistant to more than three categories of antimicrobial agents (MDR), while 27 isolates (87%) were resistant to more than three categories of antimicrobial agents and also be resistant to carbapenems (XDR *Acinetobacter*) and 10 isolates(32.25%) were non-susceptibility to all classes of antibiotics (PDR *Acinetobacter spp*.) (Table 2 and 3). The results above are in a good agreement with the results of Kumar *et al*., were tested 45 strains of *A. baumannii* , 95% (43/45) of them were multidrug-resistant (MDR) (25). In another study performed by *Begum et al*., 100% (91/91) of the clinical isolates of *A. baumannii* were found resistant to most of the antibiotics and were considered as multi-drug resistant (26).

Table 2. Result of minimal inhibitory concentration (MIC) of Antimicrobial Susceptibility Test for isolates of Acinetobacter baumannii by Vitek2 system (MDR, XDR & PDR Acinetobacter)

		Result of(MIC) of Antimicrobial Susceptibility Test																			
No.	No.	A	AMP/	PIP/	CEF	CX	CAZ	CRO	CP	IPM	MPM	AK	GEN	TOB	CIP	LEV	NTF	TSX	MDR	XDR	PDR
1	A1	R	R	R	R	R	I	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
2	A2	R	R	R	R	R	I	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
3	A3	R	R	R	R	R	I	R	R	R	R	S	R	I	I	S	R	R	5*(+)	(+)	(-)
4	A4	R	S	I	R	R	I	R	S	S	S	S	R	I	S	S	R	R	4*(+)	(-)	(-)
5	A5	R	R	R	R	R	I	R	R	R	R	R	R	I	I	S	R	R	5*(+)	(+)	(-)
6	A6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	6*(+)	(+)	(-)
7	A7	R	R	R	R	R	I	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
8	A8	R	R	R	R	R	I	R	R	R	R	I	I	I	I	S	R	R	4*(+)	(+)	(-)
9	A9	R	R	R	R	R	R	R	R	R	R	R	R	I	R	S	R	R	6*(+)	(+)	(-)
10	A10	R	I	R	R	R	I	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
11	A11	R	I	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	6*(+)	(+)	(-)
12	A12	R	R	R	R	R	R	R	R	R	R	R	R	R	I	S	R	R	5*(+)	(+)	(-)
13	A13	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	R	R	5*(+)	(+)	(-)
14	A14	R	R	R	R	R	R	R	R	S	S	I	S	I	R	R	R	R	5*(+)	(-)	(-)
15	A15	R	R	R	R	R	R	R	I	S	S	I	S	I	R	I	R	R	5*(+)	(-)	(-)
16	A16	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	6*(+)	(+)	(-)
17	A17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	6*(+)	(+)	(-)
18	A18	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	6*(+)	(+)	(-)
19	A19	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
20	A20	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
21	A21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
22	A22	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	6*(+)	(+)	(-)
23	A23	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	6*(+)	(+)	(-)
24	A24	R	R	R	R	R	R	I	R	R	R	I	I	I	I	S	R	R	4*(+)	(+)	(-)
25	A25	R	R	R	R	R	I	I	R	R	R	I	I	I	I	S	R	R	4*(+)	(+)	(-)
26	A26	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
27	A27	R	R	R	R	R	R	R	R	R	R	R	R	R	I	S	R	R	5*(+)	(+)	(-)
28	A28	R	R	R	R	R	R	R	R	R	R	I	R	I	S	S	R	R	5*(+)	(+)	(-)
29	A29	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	6*(+)	(+)	(+)
30	A30	R	R	R	R	R	R	R	R	R	R	R	R	R	R	I	R	R	6*(+)	(+)	(+)
31	A31	R	R	R	R	R	R	R	R	S	S	S	S	I	R	R	R	R	5*(+)	(-)	(-)
No.& % R		31 (10	28 (90.3)	30 (96.7)	31 (100)	31 (100)	22 (71)	29 (93.5)	29 (93.5)	27 (87)	27 (87)	21 (67.7)	25 (80.6)	20 (64.5)	21 (67.7)	3 (9.6)	31 (100)	31 (100%)	(100%)	(87%)	10 (32.25
No.& % S		0	1(3.2)	0	0	0	0	0	1 (3.2)	0	0	4 (12.9)	3 (9.6)	0	3 (9.6)	18 (58)	0	0			
No.& % I		.	2 (6.4)	1 (3.2)	0	0	9 (29)	2 (6.4)	1 (3.2)	4 (13)	4 (13)	6 (19.35)	3 (9.6)	11 (35.5)	7 (22.5)	10 (32.2)	.	.			

Table 3. Number of *A. baumannii* resistance to more than three classes of antibiotics

	Resistance to more than three classes of antibiotics			
No.	4	5	6	**Total**
Resistant *A. baumannii* Isolates	4	9	18	31

Multi-drug resistant and extensive resistant isolates of *A. baumannii* are increasingly reported globally, with the incidence range from 75% (Spain) up to 100% (Italy, Greece, Turkey, Bulgaria). In other countries, there was also a high percentage of MDR *A. baumannii* and it ranges from 96.1% in Serbia to 100% in Croatia (27). A considerable number of MDR *A.banumanii* infections of patients who treated in army healthcare centers, show to be an eventual phenomenon, no evident post-Gulf and before the year 2003, *Acinetobacter* species were healed from traumatic wound infections or healthcare-related infections at army units' hospitals in the USA. Take for instance, in 2002, only three *A. baumannii* clinical isolates were at Landstuhl Medical Center (LMC) and 11 at Walter Reed Medical Center(WRMC). The isolates number successively increased at military medical facilities (LMC, n=12, WRMC,n=41) six weeks after initiate combat operation in Iraq (28). In another study performed by Petersen *et al*., was entitled" Trauma-related Infections in Battlefield Casualties From Iraq", *Acinetobacter* species (36%) were the predominant organisms followed by *Escherichia coli* and *Pseudomonas* species (14% each) (29).

Countries of the Arabian Gulf represent a very unique situation in the epidemiology of MDR pathogens. The Gulf countries are increasingly important trade and tourist hubs, as well as sources and, at the same time, targets of medical tourism. Specific data are sparse, they indicate that *A. baumannii* is an increasing problem in the region. It was the most common (40.9%) of nosocomial MDR isolates from ICUs in Riyadh Military Hospital, more common than *K. pneumoniae* (19%) and *P. aeruginosa* (16%) (30). Furthermore, among the causative agents of bacteremia in Hamad General Hospital, Qatar 50% of all *Acinetobacter spp.* were found to be MDR (31). In a separate study were conducted in northern Iraq; 21 of *A. baumannii* from clinical specimens were identified then test their susceptibilities to different antimicrobial agents by using Vitek 2 system. The MICs of Imipenem for the resistant *A. baumannii* were ≥16, and all isolates showed multidrug resistance to various antimicrobial agents used (32).

There are many types of research of multidrug-resistant *A. baumannii* (ranged 65-100%)from hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan, Iran, Korea and from areas in the South Pacific. These MDR *A. baumannii* often spread to cause outbreaks throughout the world (27, 33). Furthermore, increased attention from specimens of UK and U.S military and nonmilitary personnel returning from operations in Iraq and Afghanistan with infections caused by MDR *A. baumannii* (34). The study result of XDR *Acinetobacter* in Ramadi Teaching Hospital showed that the Carbapenem-resistant *Acinetobacter baumannii* (CRAB) increased from 27% in a study by Nassar, (35) in 2010 to 87% in the present study (Table 2). Additionally, the results show that increasing CRAB was significantly related to increasing the use of anti-pseudomonal carbapenems (Imipenem). Other researchers found that the increase of carbapenem resistance among *Acinetobacter* species ranged from 2% to 26% in Asian/Pacific nations, 14.1% (in 2003) to 46.3% (in 2008) in Taiwan and 6% to 52% in Western countries (36).

Also, the results of this paper showed elevate burden of CRAB in Ramadi Teaching Hospital than other the results of previous studies. Several research papers found that infection resulted from CRAB was related to higher mortality and morbidity (37, 38). One of the main medical problems is controlling the CARB spread. The literature shows that CRAB acquisition risk factors were longer hospital stays, more prolonged ICU stays invasive procedures, admission to award with a high density of patients infected with CRAB, and previous exposure to antibiotics (39, 40, 41). The reasons for the present study are similar. Practically, some human-related factors are taking a crucial role in the emergence of carbapenem resistance. These are fundamentally: (i) inappropriate antibiotic prescription related to the absolute general access to antibiotics in some countries with harmful sales regulations; (ii) once carbapenem resistance has emerged, the deficiency of infection control methods in healthcare management is progress; (iii) the use of secondary therapeutic doses of antibiotics for the reinforcement of animal growth in the agricultural field (42).

Lacking data were used for the prevalence of MBLs in *A. baumannii* in Iraq, but the proportion of isolates producing MBLs in the present study (90.32%, Table 1) is in agreement with the results of a previous study done in Iran via Irfan *et al*., which were (96.6%). Therefore, the production of MBLs can be considered as a significant factor for imipenem resistance among *Acinetobacter* species in Iraq and Iran. This result also gives the proof that acquired MBLs can rapidly appear and establish a status of endemicity in certain epidemiological positions (43).

The results of MBLs producing *Acinetobacter baumannii* in this paper proved that the frequency of these isolates are increasing dramatically and emergence is a severe epidemic risk at least two reasons. Firstly, the MBLs gives not only the resistance to IPM but all beta-lactams and other classes of antibiotics such as aminoglycosides, and secondly, the genes encoding these enzymes are carried by integrating that can be transmitted horizontally to other strains (11). The 2007 Infection Control Fact Sheet for Hospital alluded to potential hazard factors for acquiring MBLs as relatively prolonged hospitalization; pre-antimicrobial therapy, treatment in the intensive care unit, haematology, burn patients where the use of antibiotics is high (44). In this research also, we notified that all MBLs from the burns ward and intensive care units were all hospitalized for more than 8 days and previous use of antibiotics. In another paper, graft application and surgical intervention were significant risk factors for MBL producers compared to non-MBL producers (45, 46).

The appearance of MBL producing isolates in intensive care units is of apparent concern and reflects the overuse of carbapenems. The inevitable use of selection pressure for the use of broad-spectrum antibiotics in intensive care units, leading to the limitation of competitive flora and the selection of multidrug-resistant strains. Therefore a rigid project against antibiotics in intensive care rooms should be adapted to prevent the further prevalence of MBLs. Doctors should determine antibiotics with wise manner. Proper implementations of proper infection control practices reduce, removes and prevent the establishment of antibiotic-resistant bacteria such as dominant microorganisms in the burn unit and prevent cross-contamination (44, 45).

Antibiotic resistance pattern for the present study shows that all isolates of *A. baumannii* exhibited a high rate of resistance (100%) to Ampicillin, Cefazolin, Cefoxitin, Nitrofurantoin, and Trimethoprim/sulfamethoxazole. Most isolates (96.7%) were resistance to Piperacillin/Tazobactam. Resistance to anther antibiotics varied among isolates of *A. baumannii*, were (93.5%) for Ceftriaxone and Cefepime, (90.3%) for Ampicillin/Sulbactam, (87%) for Imipenem and meropenem, (80.6%) for Gentamicin, (71%) for Ceftazidime, (67.7%) for Amikacin and Ciprofloxacin and (64.5%) for Tobramycin, while the lowest was to Levofloxacin(9.6%) (Table 2).

The problems of antimicrobial resistance among *A. baumannii* isolates can be divided to the following three main categories; First, antibacterial-inactivating enzymes, second, reduced access to bacterial targets (due to decreased outer membrane permeability caused by the loss or reduced expression of porins, overexpression of multidrug efflux pumps) and third, mutations that change targets or cellular functions (alterations in penicillin-binding proteins; PBPs). Many combined mechanisms can be present in the same bacteria. Same results have also been noticed in other gram-negative bacteria (47). Statistical analysis using Chi-Square for the result of minimal inhibitory concentration (MIC) of antimicrobial susceptibility test for *Acinetobacter baumannii* isolates in the present study show a significant difference ('P' < 0.05) for Levofloxacin and Ceftazidime (Table 4). Out of 31isolates, 18 (58%) isolates were sensitive to Levofloxacin. This result is in agreement with the results of Huang *et al*., 5 isolates of *Acinetobacter baumannii* (5/11, 45%) were sensitive to levofloxacin (48). For treatment MBLs producing *Acinetobacter baumannii*, limited choices are useful and the only pharmaceutical option may be polymyxins, but it should not be used as monotherapy. It can be bind to an appropriate aminoglycoside. Synergistic treatment is oftentimes used in the treatment of MDR *Acinetobacter baumannii*. Imipenem or meropenem bind to ampicillin-sulbactam is active against carbapenem-resistant as well as MBL-positive strains of *Acinetobacter* species (44).

Table 4. Chi-Square for the result of minimal inhibitory concentration (MIC) of Antimicrobial Susceptibility Test for isolates of *Acinetobacter baumannii* by Vitek2 system

No.	LEV	CIP	TOB	GEN	AK	MPM	IPM	CP	CRO	CAZ	PIP/TAZ	AMP/ SUL
No.&% R	3 (9.6)	21 (67.7)	20 (64.5)	25 (80.6)	21 (67.7)	27 (87)	27 (87)	29 (93.5)	29 (93.5)	22 (71)	30 (96.7)	28 (90.3)
No.&% S	18 (58)	3 (9.6)	0	3 (9.6)	4 (12.9)	0	0	1 (3.2)	0	0	0	1 (3.2)
No.&% I	10 (32.2)	7 (22.5)	11 (35.5)	3(9.6)	6 (19.35)	4 (13)	4 (13)	1 (3.2)	2 (6.4)	9 (29)	1 (3.2)	2 (6.4)
Chi-Square	10.903	17.290	2.613	31.226	16.710	17.065	17.065	50.581	23.516	5.452	27.129	45.355
df	2	2	1	2	2	1	1	2	1	1	1	2
Asymp. sig.	.004*	.000	.106	.000	.000	.000	.000	.000	.000	.020*	.000	.000

***** Significant difference ('P' < 0.05),LEV: Levofloxacin, CIP: Ciprofloxacin, TOB: Tobramycin, GEN: Gentamicin, AK: Amikacin, MPM: Meropenem, IPM: Imipenem, CP: Cefepime, CRO: Ceftriaxone, CAZ: Ceftazidime, PIP/TAZ: Piperacillin/Tazobactam, AMP/SUL: Ampicillin/Sulbactam, R: Resistant, I: Intermediate and S: Sensitive.

CONCLUSION

Prevalence of Multi-drug resistant and extensive resistant *Acinetobacter baumannii* isolates from clinical specimens. It was found that the majority of *Acinetobacter baumannii* isolates were Metallo beta-lactamase (MBL) producers using IPM-EDTA-disk synergy test. Also, it is the principal mechanism of resistance among Iraqi nosocomial isolates of *A. baumannii.*

Conflict of interest

No conflict of interest was declared by the authors.

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