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 İlaça ve Kapsamlı Dirençli Acinetobacter Baumannii İzolatlarının Antibiyotik Direnç Modeli ve Prevalansı Batı Irak

Ahmed Saadoun Jaloot¹, Mustafa Nadhim Owaid¹²

¹ Department of Environmental Sciences, College of Applied Sciences-Heet, University of Anbar, Hit, Anbar, Iraq
² Department of Het Education, General Directorate of Education in Anbar, Ministry of Education, Hit, Anbar, Iraq

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 were 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 isolates (87%) were resistance to Imipenem by MICs obtained by VITEK-2. Results reported that all isolates (31/31) were Ampicillin/Sulbactam 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 Amoxicillin, 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 Cefazadime, (67.7%) for Amikacin and Ciprofloxacin and (64.5%) for Tobramycin. Furthermore, the lowest resistance was to Levofloxacine (9.6%), while 58% of isolates (18/31) were sensitive to Levofloxacine.

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 batisındaki Askeri Operasyonlar sonrasında Ramadi Eğitim Hastanesi’nde klinik örneklerden izole edilen A. baumannii izolatları arasındaki çok ilaca dirençli araştırılmaktır. Nisan 2011-Haziran 2012 döneminde kültür pozitif olan toplam hasta sayısı iki yüz on üçten seksen ikiyedi (%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 çok ilaca dirençli A. baumannii prevalansını için Vitek2 sistemi tarafından antimikrobial duyarlılık testinin minimal inhibitör konsantrasyonu (MIC) kullanıldı. A. baumannii’nin otuz bir izolatından ayrılmış seçili (90.32%) IPM-EDTA-disk sinerji testi (pozitif) kullanılarak MBL üreticisi olarak belirlenildi. Antibiyotik direnç paterni, tüm izolatların Ampisilin, Sefazolin, Sefoksitin, Nitrofurantoin ve Trimetoprim/sulfametoksazol karşı yüksek oranda (%100) direnç sergilediği göstergi. Izolatların çoğu (%9.6) Piperacillín/Tazobaktam’a dirençliydi. Anter antibiyotiklerle direnç A. baumannii izolatları arasında farklılık göstermekle birlikte, Ceftriaxone ve Cefepime için (%93.5), Ampisilin/Sulbaktam için (%90.3), Imipenem ve meropenem için (%87), Gentamisin için (%90.6), (%71) idi.) Sefazidim için (%67.7) Amikasin ve Siprofloksasin için (%64.5) Toberlamisin için. Ayrıca en düşük direnç Levofloksasine (%9.6) karşı iken, izolatların %58’i (18/31) Levofloksasine duyarlıydı.

Anahtar Sözcüklær: Çok ilaca dirençli Acinetobacter baumannii, Metallo-beta-laktamaz üretim, Carbapenem dirençli Acinetobacter baumannii

Geliş Tarihi: 07.01.2020 Kabul Tarihi: 26.05.2020

ORCID IDs: A.S.J. 0000-0002-8753-4333, M.N.O. 0000-0001-9005-4368

Address for Correspondence / Yazışma Adresi: Mustafa Nadhim Owaid, MD Department of Environmental Sciences, College of Applied Sciences-Heet, University of Anbar, Hit, Anbar, Iraq

©Telêf Hakki 2021 Gazi Üniversitesi Tip Fakültesi - Makale metnine http://medicaljournal.gazi.edu.tr/ web adresinden ulaşılabilir.

©Copyright 2021 by Gazi University Medical Faculty - Available on-line at web site http://medicaljournal.gazi.edu.tr/

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 recent 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%).
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 1 No. of Acinetobacter baumannii isolates as to the sex and type of specimen

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. of Isolates | Testing For metallo beta-lactamase production (mm) | Result of MIC For IPM by Vitek2 system |
|-----------------|--------------------------------------------------|--------------------------------------|
|                 | IPM+EDTA | IPM |                    |                                    |
| 1               | 25       | 10  | R                  | R+                                  |
| 2               | 20       | 12  | R                  | R+                                  |
| 3               | 25       | 9   | R                  | R+                                  |
| 4               | 27       | 9   | R                  | S+                                  |
| 5               | 19       | 11  | R                  | R+                                  |
| 6               | 26       | 10  | R                  | R+                                  |
| 7               | 26       | 6   | R                  | R+                                  |
| 8               | 29       | 10  | R                  | R+                                  |
| 9               | 26       | 11  | R                  | R+                                  |
| 10              | 20       | 12  | R                  | R+                                  |
| 11              | 20       | 12  | R                  | R+                                  |
| 12              | 21       | 11  | R                  | R+                                  |
| 13              | 25       | 11  | R                  | R+                                  |
| 14              | 26       | 26  | S                  | S+                                  |
| 15              | 27       | 27  | S                  | S+                                  |
| 16              | 26       | 9   | R                  | R+                                  |
| 17              | 26       | 9   | R                  | R+                                  |
| 18              | 25       | 10  | R                  | R+                                  |
| 19              | 26       | 9   | R                  | R+                                  |
| 20              | 24       | 10  | R                  | R+                                  |
| 21              | 24       | 10  | R                  | R+                                  |
| 22              | 25       | 9   | R                  | R+                                  |
| 23              | 23       | 10  | R                  | R+                                  |
| 24              | 24       | 10  | R                  | R+                                  |
| 25              | 24       | 10  | R                  | R+                                  |
| 26              | 23       | 10  | R                  | R+                                  |
| 27              | 25       | 10  | R                  | R+                                  |
| 28              | 24       | 11  | R                  | R+                                  |
| 29              | 23       | 9   | R                  | R+                                  |
| 30              | 25       | 10  | R                  | R+                                  |
| 31              | 26       | 25  | S                  | S+                                  |

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 spp.) 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).
| No. | No. | A   | AMP/ | PIP/ | CEF | CK  | CAZ  | CRD  | CP   | IPM | MPM | AK   | GEN | TOB  | CIP  | LEV | NTF | TSX |
|-----|-----|-----|------|------|-----|-----|------|------|------|-----|-----|------|-----|------|------|-----|-----|-----|
| 1   | A1  | R   | R    | R    | R   | R   | R    | R    | R    | R   | R   | R    | R   | R    | R    | R   | R   | R   |
| 2   | A2  | R   | R    | R    | R   | R   | R    | R    | R    | R   | R   | R    | R   | R    | R    | R   | R   | R   |
| 3   | A3  | R   | R    | R    | R   | R   | R    | R    | R    | R   | S   | R    | I    | I    | S    | R   | R   | R   |
| 4   | A4  | R   | S    | I    | R    | R   | R    | S    | S    | S   | S   | R    | I    | S    | S    | R   | R   | R   |
| 5   | A5  | R   | R    | R    | R    | R   | R    | R    | R    | R   | R   | R    | R   | R    | R    | R   | R   | R   |
| 6   | A6  | R   | R    | R    | R    | R    | R    | R    | R    | R   | R    | R    | R    | R    | S    | R   | R   | R   |
| 7   | A7  | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R   | R   | R   |
| 8   | A8  | R   | R    | R    | R    | R    | R    | R    | I    | I    | I    | I    | I    | I    | S    | R   | R   | R   |
| 9   | A9  | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | S    | R   |
| 10  | A10 | R   | I    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | R    | R   |
| 11  | A11 | R   | I    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    | R   | R   |
| 12  | A12 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    | R   |
| 13  | A13 | R   | R    | R    | R    | R    | R    | R    | R    | R    | S    | R    | R    | R    | S    | S    | R   | R   |
| 14  | A14 | R   | R    | R    | R    | R    | R    | R    | R    | R    | S    | S    | S    | I    | S    | I    | R    | R   |
| 15  | A15 | R   | R    | R    | R    | R    | R    | R    | I    | S    | S    | S    | I    | S    | I    | R    | I    | R   |
| 16  | A16 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    | R    | R   |
| 17  | A17 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    | R    | R   |
| 18  | A18 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    | R   |
| 19  | A19 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    | R   |
| 20  | A20 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | R    | R   |
| 21  | A21 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | R   |
| 22  | A22 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    |
| 23  | A23 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | S    | R    | R   |
| 24  | A24 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R   |
| 25  | A25 | R   | R    | R    | R    | R    | R    | R    | I    | I    | I    | R    | R    | I    | I    | I    | I    | S    |
| 26  | A26 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | R    |
| 27  | A27 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | S    | R    |
| 28  | A28 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | R    | I    |
| 29  | A29 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    |
| 30  | A30 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | I    | R    |
| 31  | A31 | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    |

Table 2. Result of minimal inhibitory concentration (MIC) of Antimicrobial Susceptibility Test for isolates of Acinetobacter baumannii by Vitek 2 system (MDR, XDR & PDR Acinetobacter)
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. _baumannii_ 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 present 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 ward 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 by 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 another antibiotics varied among isolates of _A. baumannii_, were (93.3%) for Ceftiraxone 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 31 isolates, 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 | MP | IPM | CP | CRO | CAZ | PIP/TAZ | AMP/SUL |
|-----|-----|-----|-----|-----|----|----|-----|----|-----|-----|---------|---------|
| No.&% R | 3   | 21  | 20  | 25  | 21 | 27 | 29  | 29 | 30  | 28  |         |         |
|      | (9.6) | (67.7) | (64.5) | (80.6) | (67.7) | (87) | (87) | (93.5) | (93.5) | (71) | (96.7) | (90.3) |
| No.&% S | 18  | 3   | 0   | 3   | 4  | 0  | 1   | 0  | 0   | 1   |         |         |
|      | (58) | (9.6) | (9.6) | (12.9) |     | (3.2) | (3.2) |     |     |     |         |         |
| No.&% I | 10  | 7   | 11  | 3(9.6) | 6  | 4  | 4   | 1  | 2   | 9   | 1       | 2       |
|      | (32.2) | (22.5) | (35.5) | (19.35) | (13) | (13) | (3.2) | (6.4) | (29) | (3.2) | (6.4)   |
| Chi-Square df | 65  |     |     |     |     |     |     |     |     |     |         |         |
| Asymp. sig. | 0.004* | 0.000 | 0.106 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.020* | 0.000 | 0.000   |

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

CONCLUSION

Prevalence of multi-drug resistant and extensive resistant Acinetobacter baumannii isolates was found 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.

REFERENCES

1. Jaloot AS, Al-Ouqaili MT, Badawy AS. Molecular and Bacteriological Detection of Multi-drug resistant and Metallo-β-Lactamase Producer Acinetobacter baumannii in Ramadi City, West of Iraq. Al-Anbar Med J. 2016; 13:1-13.
2. Asf M, Alvi IA, Rehman SU. Insight into Acinetobacter baumannii pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Res. 2018; 11:1249–60.
3. Dong W, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and Pathophysiological Overview of Acinetobacter Infections: a Century of Challenges. Clin Microbiol Rev. 2017; 30(1): 409-47.
4. Howard A, O’Donoghue M, Feeney A, Sleator RD. Acinetobacter baumannii: An emerging opportunistic pathogen. Vulnirurce. 2012; 3(3): 243–50.
5. Ahir HR, Patel PH, Berry RA, Parmar R, Soni ST, Shah PK, Vegad MM, Patil S. Prevalence of Metallo-β-lactamases producing Pseudomonas and Acinetobacter species in tertiary care teaching hospital, Gujarat. Int J Microbiol. Res. 2012; 4(9): 322-5.
6. Durante-Mangoni E, Zarrilli R. Global spread of drug-resistant Acinetobacter baumannii: molecular epidemiology and management of antimicrobial resistance. Futt Microbio. 2011; 6: 407–22.
7. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbio Infect. 2012;18: 268–81.
8. Almasaudi SB. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi J BioSci. 2018; 25(3): 586–96.
9. Sheu C, Chang Y, Lin S, Chen Y, Hsueh P. Infections Caused by Carbapenem-Resistant Enterobacteriaceae: An Update on Therapeutic Options. Front Microbio. 2019; 10(00): 1-13.
10. Codjoe FS, Donkor ES. Carbapenem Resistance: A Review. Med. Sci. 2018; 6(1): 1.
11. Hawkey PM, Warren RE, Livermore DM, McNulty CAM, Enoch DA, Otter JA and Wilson APR. Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party. J Antimicrob Chemother. 2018; 73(3): 2–78.
12. bioMérieux. VITEK® 2 Systems Product Information. bioMérieux, 2010; Inc. USA.
13. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA Disk Method for Differentiation of Metallo-β-Lactamase Producing Clinical Isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbio. 2002; 40(10): 3798-801.
14. Flanagan E, Cassone M, Montoya A, Mody L. Infection Control in Alternative Health Care Settings: An Update. Infect Dis Clin North Am. 2016; 30(3): 785–804.
15. Blanco N, Harris AD, Rock C, Johnson JK, Pineles L, et al. Risk Factors and Outcomes Associated with MultidrugResistant Acinetobacter baumannii upon Intensive Care Unit Admission. Antimicro Agents Chemother. 2018; 62(1): 1-7.
16. Wisplinghoff H, Perbix W, Seifert H. Risk Factors for Nosocomial Bloodstream Infections Due to Acinetobacter baumannii: A Case-Control Study of Adult Burn Patient. Clin Infect Dis. 1999; 28: 59-66.
17. Panjeshahin M, Lari A, Talei A, Shamsnia J, Alaghehbandan R. Epidemiology and mortality of burns in the South West of Iran. Burns. 2001; 27(3): 219-26.
18. Sharma MK, Bisht D, Pal S. Detection of Metallo-β-lactamase producing Gram Negative Bacteria in clinical isolates in Tertiary care Hospital - A prospective study. Int Arch Integ Med. 2019; 6(4): 107:11.
19. Clinical and laboratory standards institute “CLSI”. M100-S21. performance standard for antimicrobial susceptibility testing, twenty-first informational supplement. 2011; 31(1):1-165.
20. Ghazawi AA. Molecular epidemiological studies on sporadic and epidemic isolates of Acinetobacter baumannii. PhD Thesis. Biological Doctoral School,University Of Pécs, Hungary. 2012.
21. Kottahachchi J, Faoagali J, Kleinschmidt S. Comparison of Meropenem MIC by E Test and VITEK 2 in resistant Pseudomonas and Acinetobacter isolates. Sri Lanka J Infect Dis. 2012; 1(2): 28-35.
22. Febbraro F, Rodio DM, Puggioni G, Antonelli G, Pietropaolo V, Trancassini M. MALDI-TOF MS Versus VITEK2: Comparison of Systems for the Identification of Microorganisms Responsible for Bacteremia. Curr Microbio. 2016; 73(6): 843–50.
23. Shokri D, Khorasgani MR, Fatemi SM, Soleimani-Delfan A. Resistotyping, phenotyping and genotyping of New Delhi metallo-b-lactamase (NDM) among gram-negative bacilli from Iranian patients. J Med Microbio. 2017; 66:402–11.
24. Basak S, Singh P, Rajurkar, M. Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study. J Path. 2016; 1-5.
25. Kumar AV, Pillai VS, Dinesh KR, Karim S. The Phenotypic Detection Of Carbapenemase In Meropenen Resistant Acinetobacter Calcoaceticus–Baumannii Complex In A Tertiary Care Hospital In South India. J Clin Dia Res. 2011; 5(2): 223-6.
26. Begum S, Hasan F, Hussain S, Shah AA. Prevalence of multi drug resistant Acinetobacter baumannii in the clinical samples from Tertiary Care Hospital in Islamabad, Pakistan. Pak J Med Sci. 2013; 29(5): 1253-8.
27. Dedeić-Ljubović A, Granov B, Hukić M. Emergence of extensive drug-resistant (XDR) Acinetobacter baumannii in the Clinical Center University of Sarajevo, Bosnia and Herzegovina. Medicinski Glasnik. 2015; 12(2): 169-76.
28. Bergogne-Bérézin E, Bendinelli M, Friedman H. Acinetobacter Biology and Pathogenesis. Springer.com. New York,USA. 2008.
29. Petersen K, Riddle MS et al. Trauma-related infections in battlefield casualties from Iraq. Ann Surg. 2007; 245: 803-11.
30. Ibrahim ME. Prevalence of Acinetobacter baumannii in Saudi Arabia: risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance. Ann Clin Microbiol Antimicrob. 2019; 18:1.
31. Al Samawi MS, Khan FY, Eldeeb Y, Almaslamani M, Alkal A, Alsob H, Ghabdan, W, Howady F, Hashim S. Acinetobacter Infections among Adult Patients in Qatar: A 2-Year Hospital-Based Study. Can J Infect Dis Med Microbiol. 2016; 1-5.
32. Shali AAK. Identification of Multidrug-Resistant Genes in Acinetobacter baumannii in Sulaimani City-Kurdistan Regional Government of Iraq. Asian Journal of Medical Sciences, 2012; 4(5): 179-83.
33. Izadi B, Souzani R, Farahani A, Mehrabian S, Mohajeri P. Frequency of class 2 integrons in multidrug-resistant Acinetobacter baumannii isolated from patients in West of Iran. Ann Trop Med Pub Heal. 2017; 10(1): 104-8.
34. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN and Bonomo RA. Global Challenge of Multidrug-Resistant Acinetobacter baumannii. Antimicrob Agents Chemother. 2007; 51(10): 3471–84.
35. Nasser RMA. Antimicrobial susceptibility of Acinetobacter baumannii obtained from Al-Ramadi Teaching Hospital. MSc Thesis, College of Medicine, University of Al-Anbar, Iraq. 2011.
36. Su C-H, Wang J-T, Hsiung CA, Chien L-J, Chi C-L, Yu H-T, Chang F-Y, Chang S-C. Increase of Carbapenem-Resistant Acinetobacter baumannii Infection in Acute Care Hospitals in Taiwan: Association with Hospital Antimicrobial Usage. PLoS ONE. 2012; 7(5): 1-6.
37. Raible KM, Sen B, Law N. Bias TE, Emery CL, Ehrlich GD, Joshi SG. Molecular characterization of β-lactamase genes in clinical isolates of carbapenem-resistant Acinetobacter baumannii. Ann Clin Microbiol Antimicrob. 2017; 75:1-10.
38. Yamamoto N, Hamaguchi S, Phainuphong P, Akeda Y, et al. Rapid screening and early precautions for carbapenem-resistant Acinetobacter baumannii carriers decreased nosocomial transmission in hospital settings: a quasi-experimental study. Antimicrob Resist Infect Cont. 2019; 8:1-10.
39. Djordjevic ZM, Follic MM, Follic ND, Gajovic N, Gajovic O, Jankovic SM. Risk factors for hospital infections caused by carbapenem-resistant Acinetobacter baumannii. J Infect Dev Ctries. 2016; 10(10):1073-80.
40. Al-Ghetamy MM, Faidah HS, Adetunji HM, Haseeb A, et al. Risk factors associated with multi-drug-resistant Acinetobacter baumannii nosocomial infections at a tertiary care hospital in Makkah, Saudi Arabia - a matched case–control study. J Int Med Res. 2017; 45(3) 1181–9.
41. Aljindan R, Elhadi N. Genetic Relationship of Multi-Resistant Acinetobacter baumannii Isolates in Kingdom of Saudi Arabia. J Pure Appl Microbiol, 2018; 12(4):1351-8.
42. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Therap Adv Infect Dis. 2016; 3(1): 15-21.
43. Irfan S, Zafar A, Guhar D, Ahsan T and Hasan R. Metallo-β-Lactamase-Producing Clinical Isolates Of Acinetobacter Species and Pseudomonas aeruginosa from Intensive Care Unit Patients of a Tertiary Care Hospital. Indian J Med Microbiol. 2008; 26(3): 243-5.
44. De AS, Kumar SH, Baveja SM. Prevalence of metallo-β-lactamase producing Pseudomonas aeruginosa and Acinetobacter species in intensive care areas in a tertiary care hospital. Indian J Crit Care Med. 2010; 14(4): 217-9.
45. Kumar SH, De AS, Baveja SM, Gore MA. Prevalence and Risk Factors of Metallo β-lactamase Producing Pseudomonas aeruginosa and Acinetobacter species in Burns and Surgical Wards in a Tertiary Care Hospital. J Lab Phys. 2012; 4(1): 39-42.
46. Ghasemian A, Rizi KS, Vardanjani HR, Nojoomi F. Prevalence of Clinically Isolated Metallo-beta-lactamase-producing Pseudomonas aeruginosa, Coding Genes, and Possible Risk Factors in Iran. Iran J Pathol. 2018; 13(1): 1-9.
47. Khaleedi A, Fatemeh D, Hosseini SMI, Meskini M, Esmaeili D. Antimicrobial Resistance Pattern of Acinetobacter baumannii Strains Isolated from Intensive Care Unit Patients. Med Lab J. 2018; 12(6):19-23.
48. Huang S, Lee S, Lee N, See L, Tsai M, Shieh W. Comparison of in vitro activities of levofloxacin, ciprofloxacin, cefazidime, cefepime, imipenem, and piperacillin-tazobactam against aerobic bacterial pathogens from patients with nosocomial infections. J Microbio Immuno Infect. 2007; 40: 134-40.