Isolation, molecular identification and antimicrobial susceptibility of Acinetobacter baumannii isolated from Baghdad hospitals

Kais Kassim Ghaima[1], Shurook Mohammad K. Saadedin[2], Kifah Ahmed Jassim[3]

[1, 2] Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.
[3] Ministry of health, Central Public Health Laboratory, Baghdad, Iraq.

Abstract- Increasing resistance to antimicrobial agents used in the treatment of burns and wounds infections with *Acinetobacter baumannii* strains has become an important concern. Isolation and identification of *A. baumannii* strains from patients with burns and wounds infections by conventional and molecular methods and detection of their antimicrobial susceptibility**.** Antibiotics resistance of 96 *A. baumannii* strains isolated from the samples collected between February 2015 and June 2015 were evaluated. Identification of *A. baumannii* isolates were determined by CHROMagar *Acinetobacter*¸ API 20E and automated system VITEK 2 and molecular methods by detection 16S rRNA and blaOXA-51 genes. Antimicrobial susceptibility tests were performed by agar disk diffusion and broth microdilution methods. Among 476 samples included burns and wounds infections, 96 isolates of *A.baumannii* were obtained, 65 belonged to burns and 31 from wounds. CHROMagar *Acinetobacter* had 100% sensitivity and specificity for isolation of *A. baumannii*. The PCR results showed that 16S rRNA sequence and blaOXA-51 gene exists in all *A. baumannii* collected from positive samples, and this confirmed the accuracy of other identification tests. In the present study, 87.5% of the isolates had Multi-Drug Resistance (MDR). It has been found that *A. baumannii* strains had high resistance to most antibiotics used: Cefotxime (87.5%), Ciprofloxacin (80.2%), Amikacin (79.2 %), Meropenem (75%) Imipenem (81.3%) and Pipracillin (81.3%) but low resistance to Tigecycline (11.5%) and Colistin (7.3%). The present results indicate that antimicrobial resistance of *A. baumannii* in Iraq has increased, which may very well affect the antimicrobial resistance of this organism worldwide. Therefore their early detection is essential for stimulates effective treatment in intensive care units. Detection of blaOXA-51-like gene and CHROMagar *Acinetobacter* can be used as a simple and reliable method to differentiate *A. baumannii* strains.

Index Terms- *Acinetobacter baumannii*, Antimicrobial susceptibility, molecular identification, burns, wounds.

I. INTRODUCTION

Acinetobacter baumannii is one of the most important opportunistic pathogens that cause outbreaks in hospitals and serious health care associated complications in hospitalized patients [1]. *A. baumannii* has become endemic in hospitals due to its versatile genetic machinery, which allows it to quickly evolve resistance factors, and to its remarkable ability to tolerate harsh environments [2]. During the past decade, nosocomial outbreaks of *A. baumannii* have been described with increasing frequency, occurring mostly in intensive care, burn and surgical units [3,4]**.** *A. baumannii* infections mainly affect patients with severe underlying disease, and are associated with major surgery, burns or trauma. Most studies report high overall mortality rates in patients with *A. baumannii* bacteremia and may be associated with considerable morbidity and (overall) mortality as high as 58% [5,6]. The increasing prevalence of multi-drug resistant in *A. baumannii* strains, have caused *A. baumannii* become the most important pathogen after *Pseudomonas aeruginosa* among non-fermentative gram-negative bacteria [7].

The most alarming problems encountered are the ability of this species to have different mechanisms of resistance and the emergence of strains that are resistant to all commercially available antibiotics coupled with the lack of new antimicrobial agents, this has resulted in a limited choice of antibiotics for treatment of multidrug resistant isolates of *A. baumannii* [8,9]. There were increasing number of hospital outbreaks caused by *A. baumannii* has been reported from several countries around the world. In addition, inter-hospital spread of multidrug resistant *A. baumannii* has been observed as well as spread among countries [10]. A number of resistance mechanisms to many classes of antibiotics are known to exist in *A. baumannii*, including β-lactamases, multidrug efflux pumps, aminoglycoside modifying enzymes, permeability defects, and the alteration of target sites [11].

This study was designed to isolation and identification of *A. baumannii* from burns and wounds infections of hospitalized patients in Baghdad hospitals using conventional and molecular methods, also this work aims to investigate antimicrobial susceptibility patterns among different *A. baumannii* isolates.

II. MATERIALS AND METHODS

Bacterial isolates

A total of ninety six *A. baumannii* isolates were collected from 476 clinical specimens such as burns and wounds from patients at Baghdad hospitals, Iraq during the February –July 2015 period. Bacterial isolation and identification were performed using standard laboratory methods. The isolates were

non-repetitive, meaning that each isolate was obtained from a particular patient and each patient was sampled only once. Samples were streaked across CHROMagar *Acinetobacter* (CHROMagar, France), MacConkey and blood agar plates for all specimens. Presumptive identification was done based on culture characteristics, gram stain and conventional biochemical tests [12].

Standard identification, confirmation and complete method were conducted including using API 20E (bioMérieux, France) and Vitek 2 system (bioMérieux, France) with ID-GNB card for identification of gram-negative bacilli, according to the manufacturer's instructions. Samples confirmed as *A.baumannii* were stored in Tryptic Soy Broth (Merck, Germany) containing 20 % glycerol at -20 °C and were subjected to further molecular identification. The PCR of blaOXA-51-like genes was used as a final confirmation as to the presence of *A. baumannii* species [13].

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed by agar disk diffusion, according to manufacturer instructions and Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. The applied antimicrobials were as follows: Amikacin (30µg), Gentamicin (10µg), Imipenem (10µg), Meropenem (10µg), Ceftazidime (10µg), Cefotaxime (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Tetracycline (10µg), Tigecyclin (30µg), Aztreonam (30µg), Ticarcillin - clavulanic acid (75/10µg), Oxaciillin (1µg) Piperacillin (30µg), Trimethoprim / Sulfamethoxazole (25µg) and Colistin (10µg) (MAST Diagnostic Co., UK). For Tigecycline, the Food and Drug Administration (FDA) breakpoints for Enterobacteriaceae (14mm, resistant; 19mm, susceptible) were used. The growth inhibition zones around each disk were measured. For minimal inhibitory concentration (MIC) determinations, broth microdilution method Mueller-Hinton broth was used according to the CLSI guidelines [15]. *Pseudomonas aeruginosa* ATCC- 27853 were used as quality reference strains.

MDR *A. baumannii* was defined as an intermediately-resistant or resistant isolate to more than two of the antimicrobial agents

DNA extraction

Genomic DNA was extracted by standard DNA Extraction Kit (Promega, USA) according to the manufacturer's instructions. The purity of DNA was evaluated by calculating the ratio of the absorbance at 260 and 280 nm (A260/A280), DNA concentration and 260/280 ratios are determined using a NanoDrop ND-1000.

Detection of 16s ribosomal RNA gene

Polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA was performed in a DNA thermal cycler, (Applied Biosystem, Singapore) with the following cycling program: Initial denaturation at 95ºC for 3 min, and 30 cycles of denaturation at 95ºC for 1 min, annealing at 55ºC for 1 min, extension at 72ºC for 1 min and a final extension at 72ºC for 5 min using primer sequence: [16]
Forward, 5-CAGCTCGTGTCGTGAGATGT-3
Reverse, 5-CGTAAGGGCCATGATGACTT-3

Detection of bla OXA 51 carbapenemase gene

bla OXA-51 gene was amplified as described (Woodford et al., 2006) [17]. Amplified DNA fragments were purified with Qiaquick PCR purification kits (Qiagen, USA). The PCR analysis was performed using the primer sequence:

OXA-51-like forward 5 -TAA TGC TTT GAT CGG CCT TG -3
OXA-51-like reverse 5 -TGG ATT GCA CTT CAT CTT GG -3
The amplification conditions were: initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 45 seconds, 52°C for 40 seconds, 72°C for 45 seconds, and a final elongation at 72°C for 6 minutes

Amplified fragments were separated by electrophoresis in 1% agarose gel at 70V for 2hr. Finally, fragments were stained with ethidium bromide and detected under UV light.

III. Results

Among 476 samples included burns and wounds infections, 103 samples were identified as *Acinetobacter sp.* 96 isolates (20.2 %) identified as *A. baumannii*. 65 samples were from burns and 31 samples were from wounds. The results of distribution of *A. baumannii* isolates in clinical samples based on type sample are shown in Table 1.

Table 1. Distribution of *A. baumannii* and MDR *A. baumannii* isolates in clinical samples (burns and wounds).

Sample type	No. of (%) Sample	No. of *A. baumannii* (%)	No. of MDR *A. baumannii* (%)
Burns	315 (66. 2 %)	65 (20. 6 %)	57 (18. 1 %)
wounds	161 (33. 8 %)	31 (19. 3 %)	27 (16. 8 %)
Total	476	96 (20. 2 %)	84 (17. 6 %)

Of the 96 isolates of *A.baumannii* from the clinical samples, 65 belonged to burns (20.6 %). The wounds also had a high relative proportion of *A.baumannii* isolates (19.3 %). CHROMagar Acinetobacter was used for specific isolation of *A.*

baumannii. On CHROMagar isolates of *A. baumannii* appeared as bright red colonies at 24 hour as shown in figure 1, this medium also has selectivity for MDR *A. baumannii* by adding MDR Acinetobacter screening supplement. The results of conventional biochemical tests and MacConkey agar compared to CHROMagar Acinetobacter. CHROMagar Acinetobacter recovered all 96 isolates (100%). It had 100% sensitivity and specificity for isolation of *A. baumannii*. This medium also has selectivity for multidrug resistant *A. baumannii* (MDRAB).

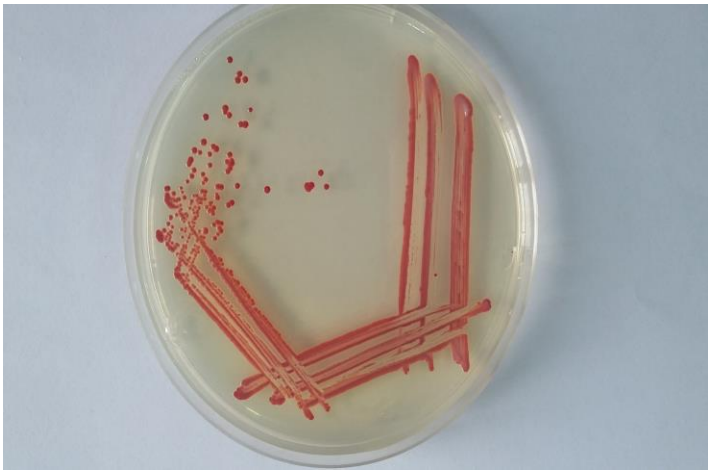

Figure 1. Colonies of *Acinetobacter baumannii* on CHROMagar Acinetobacter.

The PCR results showed that 16S rRNA sequence exists in all 103 *Acinetobacter sp.* collected positive samples, and this confirmed the accuracy of biochemical tests. The results of PCR of blaOXA-51 gene showed that this gene exists in all 96 isolates which had been diagnosed as *A. baumannii*. These results may reflect more accurate and more sensitive detection of molecular diagnosis in comparison with CHROMagar Acinetobacter and biochemical tests. Figure 2 shows the results of PCR of the 16S rRNA gene. Figure 3 shows the PCR results of the bla OXA-51gene.

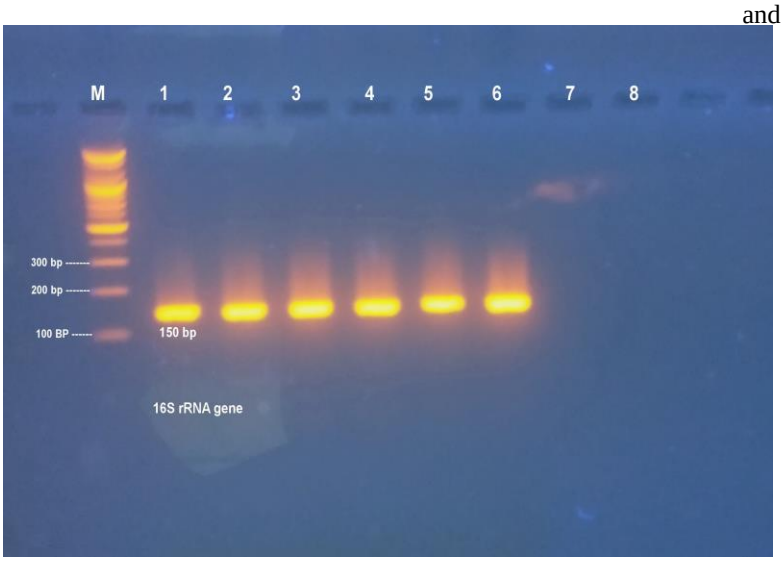

Figure 2. Ethidium bromide stained agarose gel (2%) of PCR amplified products from extracted DNA of *A. baumannii* isolates and amplified with primers of 16S rRNA gene. Lane (M): DNA molecular size marker (l00bp ladder), Lane (1-5): show a positive result with positive bands of 150bp 16S rRNA gene of *A. baumannii*, Lane (6): show a positive result for *A. lwoffii*, lane (7): Negative control, (70V for 2hr).

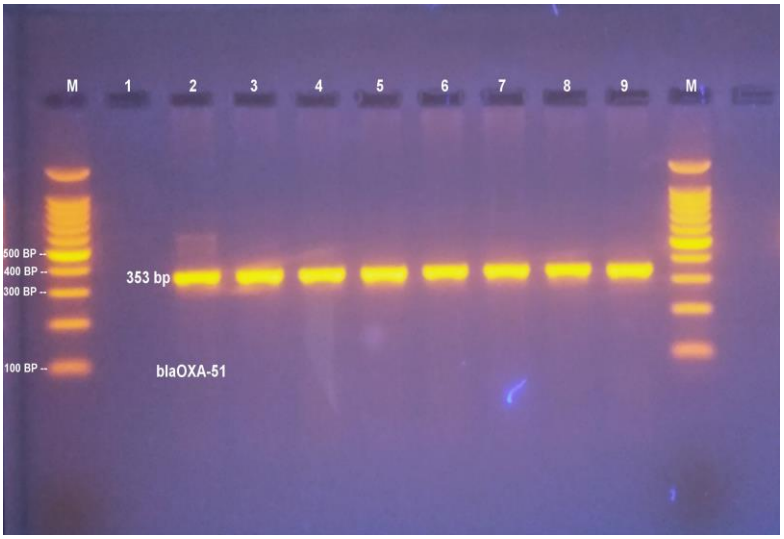

Figure 3. Ethidium bromide stained agarose gel (2%) of PCR amplified products from extracted DNA of *A. baumannii* isolates and amplified with primers of bla OXA-51 gene. Lane (M): DNA molecular size marker (l00bp ladder), lane (1): Negative control. Lane (2- 9): show a positive result with positive bands of 353bp. (70V for 2hr).

In the present study, 87.5 % of the isolates had multidrug resistance. It has been found that *A. baumannii* strains had high resistance to Cefotxime (87.5%), Imipenem (81.3%), Pipracillin (81.3%), Ciprofloxacin (80.2 %), Amikacin (79.2%) and Meropenem (75%), but low resistance to Tigecycline (11.5%) and Colistin (7.3%)

Table 2. Percentages of antimicrobial susceptibility rate of 96 *A. baumannii* isolates against 16 antimicrobial agents.

Antibiotic	Resistant	Intermediate	Sensitive
Amikacin AK	76 (79.2 %)	2 (2.1 %)	18 (18.8 %)
Gentamicin GM	75 (78.1 %)	11 (11.5 %)	10 (10.4 %)
Imipenem IPM	78 (81.3 %)	8 (8.3 %)	10 (10.4 %)
Meropenem MEM	72 (75 %)	11 (11.5 %)	13 (13.5 %)
Ceftazidime CAZ	60 (62.5 %)	11 (11.5 %)	25 (26 %)
Cefotaxime CTX	84 (87.5 %)	6 (6.3 %)	6 (6.3 %)
Ciprofloxacin CIP	77 (80.2 %)	8 (8.3 %)	11 (11.5 %)
Levofloxacin LVX	72 (75 %)	3 (3.1 %)	21 (21.9 %)
Tetracycline TE	50 (52.1 %)	0 (0 %)	46 (47.9 %)
Tigecycline TGC	11 (11.5 %)	7 (7.3 %)	78 (81.3 %)
Aztreonam ATM	74 (77.1 %)	4 (4.2 %)	18 (18.8 %)
Ticarcillin–K clavulanic acid; TCC	67 (69.8 %)	6 (6.3 %)	23 (23.9%)
Oxacillin OX	80 (83.3 %)	8 (8.3 %)	8 (8.3 %)
Piperacillin PI	78 (81.3 %)	6 (6.3 %)	12 (12.5 %)
Trimethoprim–sulphamethoxazole TS	81 (84.4 %)	10 (10.4 %)	5(5.2 %)
Colistin CO	7 (7.3 %)	5 (5.2 %)	84 (87.5 %)

IV. Discussion

The bacterium *A. baumannii* is a major hospital acquired pathogenic microorganism and usually affecting patients who are immunocompromised and those patients hospitalized in intensive care and burns units [18,19]. In this study, frequency of *A. baumannii* in burns was determined by 20.6 %. Hussein *et al*., [20] determined the frequency of *A. baumannii* by 5.22 % in burns specimens in intensive care units of Baghdad hospitals. In a study conducted by Mamani *et al*., [21] in Hamedan, frequency of *Acinetobacter* in burn wounds were about 16.6%. Previous study in Hilla hospitals including frequency of *A. baumannii* in wounds (3.12%) was lower than our results [22].

CHROMagar *Acinetobacter* is a recently developed selective agar for the rapid identification of MDRAB. It contains agents which inhibit the growth of most gram-positive organisms as well as carbapenem-susceptible gram-negative bacilli; Gordon

et al., [23] results of CHROMagar *Acinetobacter* were compared with a molecular assay resulting in sensitivity and specificity of culture compared to PCR of 91.7% and 89.6%, respectively.
16S rRNA gene used for bacterial identification that is more robust, reproducible, and accurate than that obtained by phenotypic testing, hence *Acinetobacter* clinical isolates were identified by detection the bacteria 16S rRNA genes [24,25].

blaOXA-51genes are factors of resistance to carbapenem antibiotics in *Acinetobacter* which exists inherently in all *A.baumannii* strains and is chromosomal. The existence of the bla OXA-51gene was investigated to prove *A. baumannii* strains [26,27]. The results indicated the presence of this gene in all the strains of *A.baumannii* and consistent with previous research [26-28]. Based on the results of antibiotic susceptibility (Table 2), among 96 samples of *A. baumannii*, 84 samples were resistant to more than 3 classes selected antibiotics, in other words, MDR *A. baumannii* (table 2).

The percentage of multidrug resistant isolates were in agreement with the results obtained by Ronat *et al*. [29] who found that multidrug-resistant organisms were common invasive pathogens in burn-injury patients in northern Iraq. Multiple factors have contributed to antimicrobial resistance in clinical *A. baumannii* isolates, since MDR *A. baumannii* strains have multitude of resistance genes that *A. baumannii* may possess along with the potential horizontal gene transfer between polyclonal MDR *A. baumannii* strains. The presence of class 1 integrons and ISAba1 elements are always linked to the epidemic potential of *A. baumannii* [30,31] .

Antibiotic resistance in *A.baumannii* is increasing at an alarming rate leading to increased morbidity, mortality and treatment costs in intensive care units settings as revealed by surveillance studies from over the last years [32].

V. Conclusions

These results revealed that *A. baumannii* incredibly improve resistance against antibiotics therefore the number of effective antibiotics will reduce dramatically and *A. baumannii* will cause serious health problems worldwide by time. The prevalence of *A. baumannii* infections causing the majority of burns and wounds infections is rising and the resistance against antibiotics is un-preventable globally.

References

[1] Corbella X, Montero A, Pujol M, Dominguez MA, Ayats J, Argerich MJ, et al. Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multiresistant Acinetobacter baumannii. J Clin Microbiol. 2000; 38: 4086–4095.

[2] Lin M, Lan C. Antimicrobial resistance in Acinetobacter baumannii : From bench to bedside. World J Clin Cases. 2014; 2(12): 787-814.

[3] Jafari R, Karbasizade V. Frequency and Antimicrobial Susceptibility of Acinetobacter baumannii in Burn infections in Isfahan, Iran. Advances in Bioresearch. 2014; 5 (2): 148-152.

[4] Norouzi J, Heydarpour A, Mohammadi M. Acinetobacter as nosocomial pathogens. Archive of SID. 2003; 9(24): 1-5.

[5] Falagas ME, Rafailidis PI. Attributable mortality of Acinetobacter baumannii: no longer a controversial issue. Crit Care. 2007; 11: 134.

[6] Chuang YC, Sheng WH, Li SY, Lin YC, Wang JT, et al. Influence of genospecies of Acinetobacter baumannii complex on clinical outcomes of patients with Acinetobacter bacteremia. Clin Infect Dis. 2011; 52: 352–360.

[7] Yang H, Liang L, Lin Sjia S. Isolation and characterization of a virulent bacteriophage AB1 of Acinetobacter baumannii. Biomedical Central (BMC) Microbiology.2010; 10:131-142.

[8] Lolans K, Rice TW, Munoz-Price LS, et al. Multicity outbreak of carbapenem resistant Acinetobacter baumannii isolates producing the carbapenemase OXA-40. Antimicrob Agents Chemother. 2006; 50: 2941-2945.

[9] Urban, C., Segal-Maurer, S., Rahal, J.J. Considerations in con¬trol and treatment of nosocomial infections due to multidrug resistant Acinetobacter baumannii. Clin Infect Dis.2003; 36: 1268-1274.

[10] Visca P, Seifert H, Towner KJ. Acinetobacter Infection – an Emerging Threat to Human Health. IUBMB Life. 2011; 63(12): 1048–1054.

[11] Roca I, Espinal P, Vila-Farrés X, Vila J. The Acinetobacter baumannii Oxymoron: Commensal Hospital Dweller Turned Pan-Drug-Resistant Menace. Front Microbiol.2012; 3: 148.

[12] Constantiniu S, Romaniuc A, Smaranda L, Raluca I, Tara FI. Cultural and biochemical characteristics of Acinetobacter spp. strains isolated from hospital units. The journal of preventive medicine.2004; 12 (3-4): 35-42.

[13] Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbio.2006; 44(8):2974-6.

[14] Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests, 9th ed. Approved standard M2-A9. Clinical and Laboratory Standards Institute, Wayne, PA; 2006.

[15] Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 21st informational supplement, CLSI M100-S21, Clinical and Laboratory Standards Institute Wayne, PA; 2011.

[16] Higgins PG, H. Wisplinghoff H, Stefanik D, Seifert H. Selection of topoisomerase mutations and overexpression of adeB mRNA transcripts during an outbreak of Acinetobacter baumannii. J Antimicrob Chemother.2004; 54: 821–823.

[17] Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int. J. Antimicrob. Agents.2006; 27: 351–353.

[18] Chim H, Tan BH, Song C. Five-year review of infections in a burn intensive care unit: High incidence of Acinetobacter baumannii in a tropical climate. Burns.2007; 33 (8): 1008-14.

[19] Murray CK, Hospenthal DR. Acinetobacter infection in the ICU. Crit Care Clin.2008; 24: 237-248.

[20] Hussein N, Al-Mathkhury H, Sabbah M. Imipenem-Resistant Acinetobacter baumannii isolated from patients and hospitals environment in Baghdad. Iraqi J Sci.2013; 54: 803-812.

[21] Mamani M, Derakhshanfar A, Niayesh A, Hashemi SH, Yosefi MR, Zavar S. Frequency of Bacterial Burn Wounds Infection and Antimicrobial Resistance in Burn Center of Bessat Hospital of Hamedan. Journal of Surgery.2009; 17: (1).

[22] Rasha JM, Al-Warid AL, Al-Thahab IJ. Isolation and identification of Acinetobacter baumannii in Hilla city. Int. J. of Advanced Bio. Res.2014; 4(1): 4-8.

[23] Gordon NC, Wareham DW. Evaluation of CHROMagar Acinetobacter for Detection of Enteric Carriage of Multidrug-Resistant Acinetobacter baumannii in Samples from Critically Ill Patients. Journal of Clinical Microbiology. 2009; 47: 2249 -2251.

[24] Misbah S, AbuBakar S, Hassan H, et al. Antibiotic susceptibility and REP-PCR fingerprints of Acinetobacter spp. isolated from a hospital at ten years. J Hosp Infect.2004; 58: 254-261.

[25] Dortet L, Legrand P, Soussy CJ, Cattoir V. Bacterial identification, clinical significance, and antimicrobial susceptibilities of Acinetobacter ursingii and Acinetobacter schindleri, two frequently misidentified opportunistic pathogens. J Clin Microbiol.2006; 44: 4471–8.

[26] Shali A. Identification of multidrug – resistant genes in Acinetobacter baumannii in Sulaimani city Kurdistan regional government of Iraq. Asian Journal of Medical Sciences.2012; 4(5): 179-183.

[27] Giannouli M, Cuccurullo S, Crivaro V, Popolo A, Brnarardo M, Tomasone F, et al. Molecular Epidemiology of Multidrug-Resistant Acinetobacter baumannii in a Tertiary Care Hospital in Naples, Italy, Shows the Emergence of a Novel Epidemic Clone. J Clin Microbiol. 2010; 48: 1223-1230.

[28] Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of successful pathogen. Clinical Microbiology Review.2008; 21(3): 538-582.

[29] Ronat JB, Kakol J, Marwan NK, Mathilde B, et al. Highly Drug-Resistant Pathogens Implicated in Burn- Associated Bacteremia in an Iraqi Burn Care Unit. PLOS ONE.2014; 9 (8): 1-7.

[30] Bratu S, Landman D, Martin DA, et al. Correlation of antimicrobial resistance with beta-lactamases, the OmpA-like porin, and efflux pumps in clinical isolates of Acinetobacter baumannii endemic to New York City. Antimicrob Agents Chemother.2008; 52: 2999–3005.

[31] Turton JF, Kaufmann ME, Glover J, et al. Detection and typing of integrons in epidemic strains of Acinetobacter baumannii found in the United Kingdom. J Clin Microbiol.2005; 43: 3074–3082.

[32] Ko KS, Suh JY, Ki TK, et al. High rates of resistance to Colistin and Polymixin B in subgroups of Acinetobacter baumannii isolates from Korea. J Antimicrob Chemother. 2007; 60: 1163-1167.

AUTHORS

First Author – Kais Kassim Ghaima, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Al -Jadiria, Baghdad, Iraq, Email, kaiskassim@gmail.com. , Tel: 96407901450314.
Second Author – Shurook Mohammad K. Saadedin, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Al – Jadiria, Baghdad, Iraq, Email, ssaadedin@gmail.com.
Third Author – Kifah Ahmed Jassim, Ministry of health, Central Public Health Laboratory, Baghdad, Iraq. kifahphd@yahoo.com.