Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
2020, 17(3):726-732 **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2020.17.3.0726

Identification of *Acinetobacter baumannii* and Determination of MDR and XDR Strains

Noor Hussein Ahmad [1*] ***Ghada A. Mohammad*** [2]

[1]Medical laboratory technology Department, Al-Noor University College, Ninawa, Iraq.
[2]Department of Biology, College of Science, University of Mosul, Mosul, Iraq.
[*]Corresponding author: *noor.hussain@alnoor.edu.iq, Kadsbio32@uomosul.edu.iq
[*]ORCID ID: *https://orcid.org/0000-0001-9188-5657, https://orcid.org/0000-0003-1298-3350

Received 4/11/2018, Accepted 11/6/2019, Published 1/9/2020

Abstract:

The current study focuses on the bacterium *Acinetobacter baumannii* due to its importance as a nosocomial infections source in addition to its increased resistance against antibiotics. Different clinical and hospital environment samples were collected, and cultured on *A. baumannii* selective media: Leed Acinetobacter agar and Herellea agar. *A. baumannii* have been identified by traditional methods, followed by confirmation using molecular identification to detect $bla_{oxa\text{-}51\ like}$ gene which is considered a diagnostic gene since it is present in genome of all *A. baumannii* strains. The result was, nineteen bacterial isolates of *A.baumannii* were obtained, from twenty-seven suspected isolates, detection of local isolates belonging to MDR or XDR group. Results demonstrated that all local isolates are MDR and 16 isolates (84.2 %) are XDR.

Key words: *Acinetobacter baumannii*, *bla* $_{oxa\text{-}51}$ gene, MDR, XDR.

Introduction:

Acinetobacter baumannii (*A. baumannii*) is a gram negative bacteria, sometimes diagnosed as gram positive due to its resistance to alcohol when stained with gram stain, strict aerobic, non-motile but has some sort of twitching motility by polar fimbriae.

Oxidase negative, catalase positive, indole negative, positive for citrate with C/G content 47-39%, non-lactose fermenter, the optimum temperature for their growth is 33-35^{o}C, many strains are nitrate negative and do not form spores They are easy to grow on the non-fastidious culture media (1, 2)

A.baumannii bacteria have been of increasing importance for several reasons: they cause hospital infections and are responsible for 2-10% of all hospital-related infections of gram-negative bacteria, like septicemia, meningitis, UTI and others. They are an opportunistic pathogen, as well as for the acquisition of multi drug-resistant (MDR) and extensive drug-resistant (XDR) (3). This bacterium is resistant to many antibiotics, including aminoglycosides, tetracyclines, cephalosporins, ampicillins, cefotaximes, chloramphenicol, gentamycin, tobramycines, quinolones and marcolides (4, 5).

The main cause of *A.baumannii* infections is due to: speed in transformation, rapid development in resisting a wide range of antibiotics such as efflux pumps system and horizontal gene transfer, and prevalence in the dry environment of hospitals for long periods of time (6).

This study aims to investigate the prevalence of *A.baumannii* isolates by implementing special selective media, identification by conventional methods followed by detection of the *bla* $_{oxa\text{-}51}$ gene (diagnostic gene for *A.baumannii* because it is present in the genome of all its isolates), and that delimiting MDR and XDR *A.baumannii* isolates.

Materials and Methods:

Sampling: A total of 233 samples were collected from hospitals in Mosul (Al Salam Teaching Hospital, Al Khansaa Hospital, Ibn Al Athir Children's Hospital, Al-Gomhouri teaching Hospital) during the period from August 2017 to January 2018 (According to Ministry of Health Book No. 10397 dated 7/19/2017). The samples included: 166 specimens, from wounds, urine, respiratory infections(sputum), burns, CSF and 67 samples from the hospital environment(swabs),

including samples from intensive care unit, patient beds, surgical instruments and appliances, emergency lobby and baby incubators.

The specimens were transferred to the laboratory (by transport media) and cultured on selective *A. baumannii* media (Leed Acinetobacter medium (LAM) and Herellea agar medium) prepared according to (7, 8). As well as blood agar and MacConkey agar then incubated at 37°C for 24 hours under aerobic conditions.

Identification:

Cultural characteristics and microscopy:

The cells were examined microscopically to observe the staining reaction and cells arrangement.

Biochemical tests: The following tests were carried on: oxidase test, urease test, motility test, oxidation–fermentation test (O-F test), catalase test, IMViC test (indole production test, methyl red test, voges -proskauer test, citrate utilization test), triple sugar iron Agar, carbohydrate fermentation test. these tests prepared according to (9, 10).

Diagnosis using the Analytical Profile Index System (API 20E): It is a multi-test system that is clinically used for the rapid diagnosis of Enterobacteriaceae and other Gram negative bacteria and includes 20 tests arranged in a special strip.The test is conducted according to the instructions of the French manufacturer BioMerieux.

The diagnosis has been done using the API-20E system, which is an accurate test for the diagnosis of bacterial species. After 24 hours of incubation, the necessary reagents were added, positive and negative results were recorded and seven numbers (codes) were recorded.

Molecular identification: Standard strain of *A. baumannii* ATCC 19606 was obtained from Media center in Irbil government.

- **DNA Extraction:** Bacterial DNA was extracted from *A. baumanniii* using the boiling method by (11).
- **Determination of concentration and purity of extracted DNA**: to confirm the presence of DNA in our isolate,the concentration and purity of DNA extracted from the bacterial isolates of *A. baumannii* was measured by the Nanodrop device at the Research Laboratory of the Department of Life Sciences / College of Science.
- **PCR amplification:** PCR reactions was conducted to confirm the diagnosis of samples using the *bla* $_{oxa\text{-}51}$ gene.Using the *bla* $_{oxa\text{-}51}$ primers of the USA Alpha Company, the primer has a molecular size of 353 base pairs and consists of sequences:

bla $_{OXA51}$ F:5`TAATGCTTTGATCGGCCTTG 3`
bla $_{OXA51}$ R:3` TGGATTGCACTTCATCTTGG 5`

PCR mixture was prepared as the total size 25 microliters, 4 µL DNA (50 µL), primers (10 picomol), 6.5 µL Premix (2x) and 12.5µL Deionized water.

The previous prepared mixture was placed in thermal cycler, and the program was run as suggested by (11) :94°C for 5 min., 94°C for 30 sec., 55°C for 30 sec., 72°C for 90 sec (30 Cycles), 72°C for 7min.

Investigation of MDR and XDR isolates: The determination of MDR and XDR of *A. baumannii* local isolates was carried out by antibiotics susceptibility test (disc diffusion method) depending on (Kirby & Bauer 1966) using Mueller Hinton agar, and the antibiotics discs used were supplied from Mast English company, Ampicillin-Sulbactam 20 µg/ disc, Meropenem 10 µg/ disc, Cefetriaxone 30 µg/ disc, Azithromycin 15 µg/ disc, Erythromycin 15 µg/ disc, Clarithromycin 15 µg/ disc, Nalidixic acid 30 µg/ disc, Trimethoprime 5 µg/ disc, Levofloxacine 5 µg/ disc, Ciprofloxacine 5 µg/ disc, Amikacin 30 µg/ disc, Gentamicin 10 µg/ disc, Tobramycin 10 µg/ disc, Tetracycline 30 µg/ disc, Doxycycline 30 µg/ disc,

Results and Discussion:

Morphological result:

After taking the samples to the laboratory, they were cultured on *A. baumannii* selective media, the colonies of this bacteria on Leed Acinetobacter agar(LAM) showed a convex circular shape with smooth transluscent opaque with smooth edges of 1-2 mm diameter and pink color with pink to red background after 24 hours of incubation at 37°C (Fig. 1). The transformation of the color of the medium to red is due to the high alkali produced in the medium by the release of ammonium ions from complex nitrogen compounds in the medium. *A.baumannii* produces alkaline compounds when sucrose and fructose are consumed in the medium making the color of the medium red (9, 12).

A.baumannii colonies on the Herellea agar (Fig. 2) were round with smooth margins of 2-3 mm in the form of pale lavender flower, this is similar to what is described by (7, 8).

While colonies on MacConkey agar were pale, round and with a diameter of 2-3 mm because it is non- lactose fermenter and turned pink after 48 hours of incubation as in Fig. (3), and this description is consistent with (1). On blood agar, colonies were convex, gray or white color. It does

Baghdad Science Journal
P-ISSN: 2078-8665
E-ISSN: 2411-7986

not lyse blood because it does not producing the enzyme hemolysin as shown in the Fig. (4).

Other isolates were obtained in our study such as: *pseudomonase aeruginosa* (11.3%), *E.coli* (7.7%), *Klebsiella pneumonia* (7.2%), *Proteus vulgaris* (6%) and *Serratia liquefaciens* (3.8%).

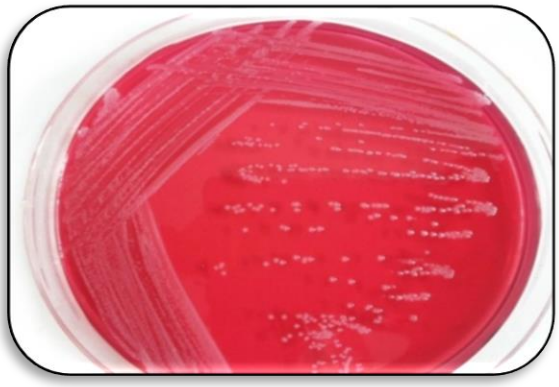

Figure 1. *A.baumannii* colonies on LAM.

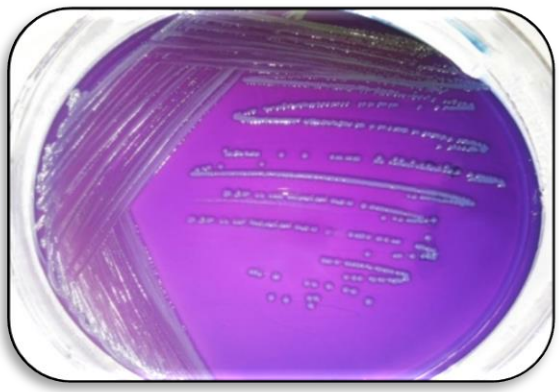

Figure 2. *A.baumannii* colonies on Herellea agar.

Figure 3. *A.baumannii* colonies on MacConkey agar.

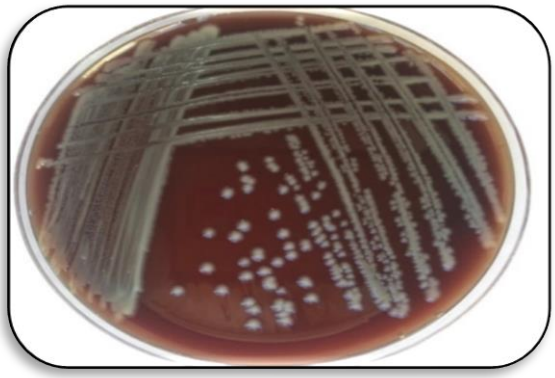

Figure 4. *A.baumannii* colonies on blood agar.

The microscopic examination of the bacterial smears showed gram negative coccobacilli, it may appear positive for gram because it is alcohol-resistant, and may be single or sometimes diplo (2).

Biochemical tests

The results of biochemical tests for identification of *A. baumannii* are shown in Table (1)**.**

Table 1. Biochemical test results for *A. baumanii*

Biochemical test		Result
Oxidase test		-
Urease test		-
O-F Test		+
Motility test		-
Catalase test		+
IMVIC		---+
Triple sugar iron agar test (TSI)		K/K, H_2S -
Sugar fermentation test	Glucose	+
	Sucrose	-
	Maltose	-
	Rabinoz	-
	Galactose	+
	Lactose	-
	Fructose	-
	Sorbitol	-

Diagnosis with API-20E:

The code was entered into the analytical profile index to find out the full scientific name for bacterial isolation, as in Fig.5.

Baghdad Science Journal
P-ISSN: 2078-8665
E-ISSN: 2411-7986

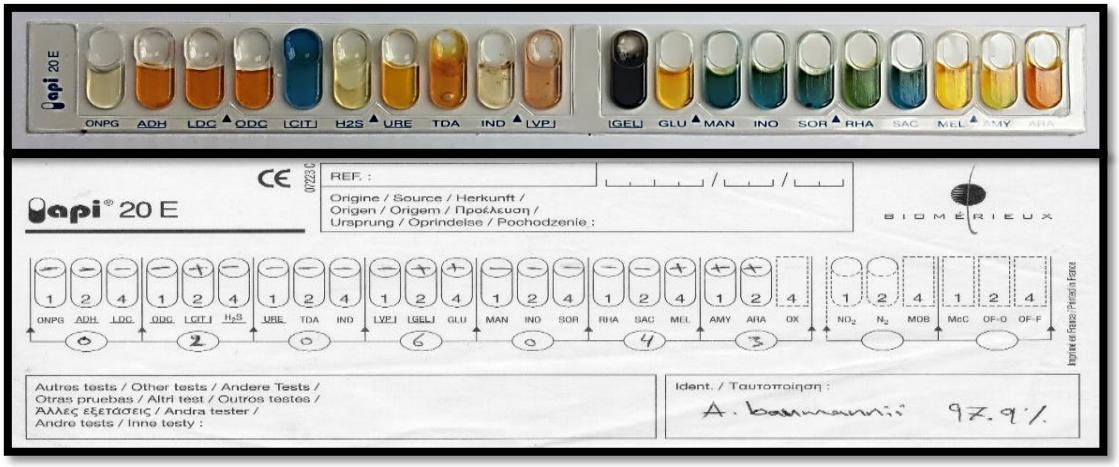

Figure 5. Result of *Acinetobacter baumannii test* by API-20E strip

Molecular diagnosis of isolates using the *bla*$_{oxa\text{-}51\ like}$ gene

After conducting the routine and API-20E identification, twenty-seven suspected isolates of *A.baumannii* have been obtained, conformation of these isolates by PCR, the result shows that 19 isolates of *A.baumannii* were obtained, whereby the appeared band by our isolates were compared by the band of a standard strain *A.baumannii* ATCC 19606 as shown in Fig. 6, 7.

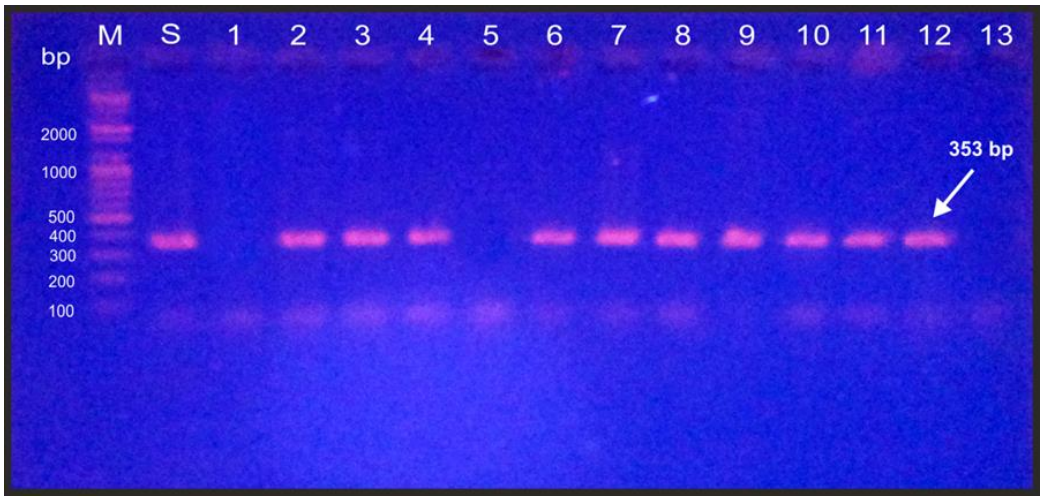

Figure 6. Gel electrophoresis for detection of *bla*$_{oxa\text{-}51\ like}$ gene in *A.baumannii* isolates on agarose gel 2% and 50 volt for 60 min. M:DNA ladder; S: standared strain. Isolates from 1-13

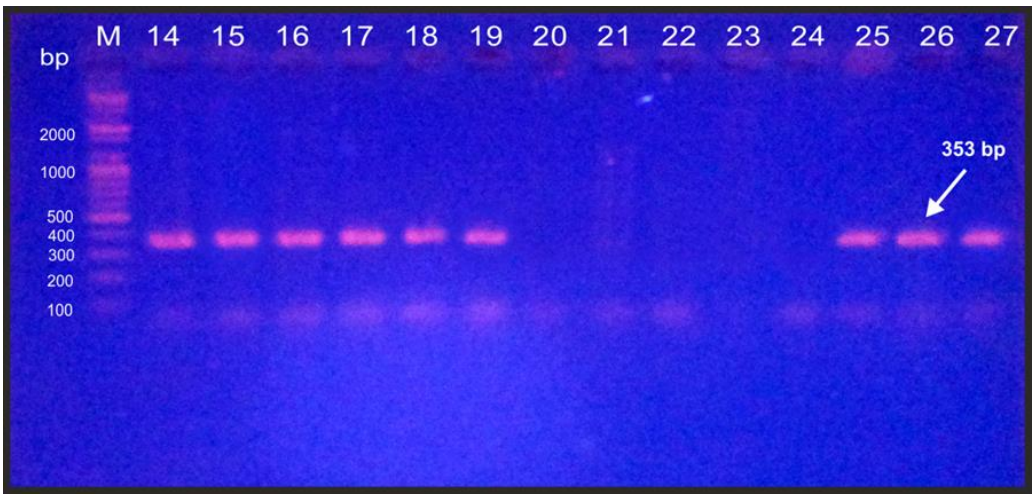

Figure 7. Gel electrophoresis for detection of *bla*$_{oxa\text{-}51\ like}$ gene in *A.baumannii* isolates on agarose gel 2% and 50 volt for 60 min. M:DNA ladder; S: standared strain. Isolates from 14-27.

Baghdad Science Journal
P-ISSN: 2078-8665
E-ISSN: 2411-7986

bla $_{oxa\text{-}51\ like}$ gene is specific for *A.baumannii* species and is present on the chromosome (13). It is a diagnostic gene of *A. baumannii* but also has a significant role in resistance to antibiotics, because it is related to the resistance of carbapenem through the production of enzymes Carbapenemases, the secondary group of these enzymes is Class D carbapenem (oxa51), also called Oxacillinase naturally located on the chromosome of this bacteria (14, 15).

The percentage of *A. baumannii* isolated in our study was 8.1 %(from 19 isolate of *A.baumannii* obtained from 233 sample), the isolates have been distributed between clinical (15) isolates and hospital environment (4) isolates (Table 1). Most isolates were from wound samples followed by respiratory tract infections, and none *A. baumannii* isolates were obtained from urine samples. The number of samples and geographical distribution of it may be the cause for different the ratio with other studies.

Table 2. Sample sources and the number of *A.baumannii* isolates.

Sample source	Total number of sample	Number of *A.baumannii* isolates
Clinical Samples		
Wounds	117	10(8.5%)
Respiratory tract infections	15	3(20%)
Burns	8	1(12.5%)
c.s.f	3	1(33.3%)
Urine	23	0
Hospital environment		
Intensive care unit	17	2(11.7%)
Patients bed	23	1(4.3%)
Devices and surgical instruments	15	1(6.6%)
Incubators	5	0
Emergency Lobby	7	0
Total	233	19(8.1%)

The results of our study agreed with the study of (16) in Baghdad as the percentage of isolation of *A.baumannii* was 8.2% compared in the present study. Also, Al-Dulaimi *et al*. (17) in Babel obtained 7% percentage of *A. baumannii* isolates from wound infections. Isolation percentage in the current work was higher than the rate of isolation of Al Sehlawi *et al*. (18) in the city of Najaf in 2014, as it was 1.9% of the total samples of wounds, but less than the isolation rate of (19) in Iran 24% isolation was obtained from respiratory infection.

Presence of MDR and XDR for isolates *A.baumannii*

For the purpose of determining which of our local isolates of *A.baumannii* belong to the MDR or XDR group, the sensitivity of the 19 bacterial isolates was first tested against 15 commonly used antibiotics. The diameter of the inhibition area was determined and compared with the tables of CLSI to determine resistance, sensitivity and moderate sensitivity (20).

Table 3 shows the ratio of sensitive isolates, resistance and average sensitivity in antibiotic susceptibility test. The results in Table (3) showed a high resistance of *A.baumannii* isolates to antibiotics, all 19 isolates were resistance 8 antibiotics(100%), while 17/19 isolates were resistance 3 antibiotics with the rate (89.4%) .

Table 3. Percentage of sensitive isolates, resistance and average sensitivity in antibiotic susceptibility test.

Antibiotic	Sensitive % isolates	Modrate isolates %	Resistance isolates |%
Ampicillin-Sulbactam	1 (5.2%)	(52.6 %) 10	8 (42.1 %)
Meropenem	____	____	19 (100 %)
Cefetriaxone	____	____	19 (100 %)
Azithromycin	____	____	19 (100%)
Erythromycin	____	2 (10.5%)	(89.4%) 17
Clarithromycin	1 (5.2%)	1 (5.2%)	(89.4 %) 17
Nalidixic acid	____	____	19 (100%)
Levofloxacine	____	2 (10.5 %)	(89.4 %) 17
Ciprofloxacine	____	____	19 (100%)
Amikacin	____	____	19 (100%)
Gentamicin	____	____	19 (100%)
Tobramycin	____	____	19 (100%)
Tetracycline	2 (10.5%)	2 (10.5%)	(78.9%) 15
Doxycycline	6 (31.5%)	1 (5.2 %)	(63.1%) 12

A.baumannii bacteria have become important and a source of study by doctors and scientists because of the rapid spread of antibiotic resistance and the slow development of new antibiotics (21).

The results of our study were consistent with the study of AL-Kadmy *et al*. (22), It showed 100% resistance of *A.baumannii* for Ciprofloxacine and Trimethoprime, the resistance to Cefetriaxone, Tobramycin, Tetracycline and B-lactams was more than 90% .While the resistance to isolates of the antibiotic Meropenem is 86% .This coincides with the results of the current study, which gave a100% resistance to this antibiotic.

Also, the results of our study were consistent with the results of the Azizi *et al*. (23) in terms of resistance to Ciprofloxacine and Gentamicin as it was 100% each of them.

When isolate was resistant to 3 classes of different antibiotics classified as MDR, the isolate was resistance of most antibiotics class except one or two groups classified as XDR

The results of our study showed 100% MDR-Ab, and that MDR was known to be at least resistant to three classes of antibiotics, such as penicillins, cephalosporins, fluroquinolones, and aminoglycosides.

Sixteen isolates (84.2%) of XDR-Ab were obtained, XDR is the isolating resistance of almost all antibiotic classes except one or two groups.

Our results matched the results of (24), with the MDR rate being 100%, and the XDR ratio of our isolates was higher (62.8%).

The MDR ratio was in agreement with that of (23).

The high resistance of isolates obtained in the present work may be due to several mechanisms: Aminoglycosides-modifying enzyme, Production of B-lactamases enzymes, Reduction in the expression of (altered) outer membrane proteins, Mutations in Topoisomerases, Work on increasing the organization of Efflux-pumps (25).

The results of the molecular diagnosis of bla $_{oxa-51}$ gene confirmed the results of our study on MDR where all isolates had this gene, and that the results of this study confirmed that XDR-Ab is the largest antimicrobial antibiotic that must be treated and controlled in hospitals and health centers (6)

Conclusion:

A.baumannii can grow on simple culture media prepared in the laboratory (non-fastidious).

It was called the term "MDR" and "XDR" because of its resistance to many antibiotics, as well as its possession of antibiotic-resistant genes such as a gene bla $_{OXA\ 51\text{-}like}$ which is a fundamental gene for the diagnosis of this type of bacteria.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mosul.

References:

1. Doughari HJ, Ndakidem PA, Human IS, Benade S. The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microbes Environ.2011; 26(2):101-112.
2. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, *et al* Biology of Acinetobacter baumannii: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol.2017;1(3): 7, 55.
3. Joly-Guillou ML. Clinical impact and pathogenicity of Acinetobacter. Clin Microbiol Infect.2005; 11(11): 868-873.
4. Gordon NC, Wareham DW. Multidrug-resistant Acinetobacter baumannii: mechanisms of virulence and resistance. Int J Antimicrob Agents.2010; 35(3): 219-226.
5. Darvishi M. Virulence factor Profile and Antimicrobial Resistance of Acinetobacter baumannii Strain isolated from various References 114 infection Recoverd from immunsuppressive patients.Biochem Pharmacol.2016; 9(3): 1057-10623.
6. Ece G, Erac B, Cetin H Y, Ece C, Baysak A. Antimicrobial susceptibility and clonal relation between Acinetobacter baumannii strains at a Tertiary Care Center in Turkey. Jundishapur J Microbiol.2015; 8(2):2-8.
7. Jawad A, Hawkey PM, Heritage J, Snelling AM. Description of Leeds Acinetobacter Medium, a new selective and differential medium for isolation of clinically important Acinetobacter spp., and comparison with Herellea agar and Holton's agar. J. Clin. Microbiol..1994;32(10): 2353-2358.
8. Atlas RM. Handbook of microbiological media 4th ed. LCC: Taylor and Francis Group; 2010. 821 p.
9. Leboffe MJ, Pierce BE. A Photographic Atlas for the Microbiology labrartory 4th ed. Morton Publishing Company;2011. P.71-98.
10. Brown A. Smith H. Benson's Microbiological Applications: laboratory manual in general microbiology, short version. 13th ed. McGraw-Hill Education.2015. P.257-261.
11. Li P, Li H, Lei H, Liu W, Zhao X, Guo L, *et al*. Rapid detection of Acinetobacter baumannii and molecular epidemiology of carbapenem-resistant *A. baumannii* in two comprehensive hospitals of Beijing, China. Front Microbiol.2015; 6(1) : 6, 997.
12. McConnell MJ, Pérez-Ordóñez A, Pérez-Romero P, Valencia R, Lepe JA, Vázquez-Barba I, *et al*. Quantitative real-time PCR for the detection of Acinetobacter baumannii colonization in the hospital environment. . J. Clin. Microbiol.. 2012;1(4):06566-11.
13. Jorgensen JH, Hindler JF, Reller LB, Weinstein MP. New consensus guidelines from the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing of infrequently isolated or

fastidious bacteria.Clin Infect Dis. 2007; 44(2): 280-286.
14. Vali L, Dashti K, Opazo-Capurro A F, Dashti AA, Al Obaid K, Evans BA. Diversity of multi-drug resistant Acinetobacter baumannii population in a major hospital in Kuwait. Front Microbiol.2015;743(6):72-76.
15. Nowak P, Paluchowska P, Budak A. Distribution of blaoxa genes among carbapenem-resistant Acinetobacter baumannii nosocomial strains in Poland. New Microbial.2012; 35: 317-325.
16. Al-Dulaimi AA, Al-Taai HR, Al-Bajlany SM. Virulence Factors of Acinetobacter baumannii isolated from different clinical specimens in Baquba. DJPS.2017;13(1): 13-26.
17. AL Mahdi ZA, Bunyan IA, AL Shukri MS. Molecular study for some virulence factors of acinetobacter baumannii isolated from patients with wound infection in hilla city. World J Pharm Res, 2016; 5(3): 175-187.
18. Al Sehlawi ZS, Al Mohana AM, Al Thahab AA. Isolation and identification of Acinetobacter baumannii clinical isolates using novel methods. JUBPAS, 2014; 22(3): 1041-1050.
19. Babapour E, Haddadi A, Mirnejad R, Angaji SA, Amirmozafari N. Biofilm formation in clinical isolates of nosocomial Acinetobacter baumannii and its relationship with multidrug resistance. Asian Pac J Trop Biomed.2016A; 6(6): 528-533
20. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing twenty-six informational supplement. Pennsylvania: Clinical Laboratory Standards Institute.2016.Chapter 36. PP. 66-67.
21. Bialvaei AZ, Samadi KH. Colistin, mechanisms and prevalence of resistance. Curr Med Res Opin.2015; 31(4):707-721.
22. AL-Kadmy IM, Ali AN, Salman IM, Khazaal SS. Molecular characterization of *Acinetobacter baumannii* isolated from Iraqi hospital environment.New Microbes New Infect. 2018; 21: 51-57.
23. Azizi O, Shahcheraghi F, Salimizand H, Modarresi F, Shakibaie MR, Mansouri S, *et al*. Molecular analysis and expression of bap gene in biofilm-forming multi-drug-resistant Acinetobacter baumannii. .Rep Biochem Mol Biol.2016; 5(1): 62.
24. Fazeli H, Taraghian A, Kamali R, Poursina F, Esfahani, BN, Moghim S. Molecular identification and antimicrobial resistance profile of Acinetobacter baumannii isolated from nosocomial infections of a teaching hospital in Isfahan, IranAvicenna J Clin Microbiol Infect.2014; 1(3).
25. Shehata AI. Phenotypic and genotypic typing of Multidrug-Resistant Acinetobacter baumannii by plasmid profiles and Multiplex-PCR typing. Sci. J. Microbiol. 2012;3(2):7.

تشخيص بكتريا *Acinetobacter baumannii* وتحديد عزلات الـ MDR وXDR

نور حسين احمد [1] **غادة عبد الرزاق محمد [2]**

[1] قسم تقنيات المختبرات الطبية، كلية النور الجامعة، نينوى، العراق.
[2] قسم علوم الحياة، كلية العلوم، جامعة الموصل، الموصل، العراق.

الخلاصة:

ركزت الدراسة الحالية على بكتريا *Acinetobacter baumannii* بسبب أهميتها كمصدر للإصابة بعدوى المستشفيات فضلا عن مقاومتها المتزايدة للمضادات الحيوية. تم جمع 233 عينة من حالات مرضية مختلفة ومن بيئة المستشفى، و زراعتها على اوساط زرعية انتقائية لبكتريا *A. baumannii* وهي: Leed Acinetobacter agar و Herellea agar. وشخصت بوساطة الطرق التقليدية ، ثم تم تأكيد التشخيص باستخدام التشخيص الجزيئي للكشف عن تواجد الجين $bla_{oxa\text{-}51\ like}$ الذي يعد جينا تشخيصيا اذ يوجد في جينوم جميع سلالات *A. baumannii*. كانت نتائج العزل والتشخيص الحصول على 19 عزلة من 27 عزلة مشكوك بها من بكتريا *A. baumannii* وأظهرت نتائج الكشف عن العزلات المحلية التابعة لمجموعة MDR او XDR بأن جميع عزلاتنا المحلية تابعة لمجموعة MDR وان 16 عزلة (84.2 %) هي XDR.

الكلمات المفتاحية: *Acinetobacter baumannii* ، *bla $_{oxa51like}$gene* ، MDR، XDR.