ISSN: 0067-2904
GIF: 0.851

Molecular Characteristics of Multidrug Resistant *Acinetobacter baumannii* Isolated from Baghdad Hospitals

Khitam K. AL-Masoudi*, Jinan M. AL-Saffar, Nuha J. Kendla
Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.

Abstract

A total of 100 clinical sample from (urine, sputum and swabs of wound , burn and ear) were collected from patients in different hospitals of Baghdad during the period from December 2013 to May 2014. 15 isolates (15%) identified belong to *Acinetobacter baumannii*, swabs of wounds were represented in high percentage of *A.baumannii* isolates (40%) while percentage of other samples were variable. Susceptibility of 15 *A.baumannii* isolates were tested toward 16 different Antimicrobial agents, the results showed all isolates were multi drug resistant. In addition, Polymerase Chain Reaction Technique (PCR) was performed to detection the resistance genes encoding the Oxacillinases enzymes. The PCR analysis showed that the presence of insertion sequence (ISAba1) in 13 isolates whereas bla$_{oxa51like}$ gene was represented in 12 isolates. Furthermore, the results of detection other genes were not appear any amplification for all *A.baumannii* isolates with genes encoded for bla *oxa 58* and bla *oxa 143* enzyme.

Keywords: Antimicrobial agent, *Acinetobacter baumannii*, OXA 51.

الخصائص الجزيئية لبكتريا *Acinetobacter baumannii* متعدده المقاومة للمضادات الحيوية والمعزولة من مستشفيات بغداد

ختام خالص المسعودي*، جنان محمد الصفار، نهى جوزيف قندلا
قسم التقنيات الاحيائيه، كليه العلوم، جامعه بغداد،العراق.

الخلاصة:

شملت الدراسة 100 عينه سريريه ومن مصادر مختلفة (الإدرار،القشع،مسحات الجروح،مسحات الحروق ومسحات الإذن) من مرضى مستشفيات مختلفة في بغداد، خلال الفترة الممتدة من كانون الأول 2013 حتى مايس 2014.،شخصت 15 عزله(15%) تعود إلى *Acinetobacter baumannii* وكانت أعلى نسبه تشخيص تعود إلى مسحات الجروح(40%) وتغايرت نسبه العزل في مصادر العينات الأخرى.اختبرت حساسية عزلات بكتريا *Acinetobacter baumannii* باتجاه 16 مضاد حيوي، أظهرت نتائج الاختبار أن جميع العزلات كانت متعدده المقاومةللمضادات الحيويه. استخدم تقنيه Polymerase Chain Reaction Technique (PCR) للتحري عن امتلاك العزلات قيد الدراسة على الجينات التي تشفر إلى (Oxacillinase)g فوجد 13 عزله تشفر للتسلسل ISAba1، بينما 12 عزله كانت تحتوي على bla $_{oxa\ 51}$ gene إما bla $_{oxa\ 143}$ gene and bla $_{oxa58}$ أظهرت نتيجة سالبه لكل العزلات.

Introduction

*Email:khitam_msc@yahoo.com

Acinetobacter baumannii is an aerobic, Gram-negative, coccobacilli, non-lactose fermenting bacterium, which has recently emerged as an important opportunistic pathogen causing nosocomial infections, including pneumonia, septicemia, and urinary tract and wound infections, and is also frequently involved in outbreaks[1]. *A. baumannii* are frequently resistant to the drug families such as aminoglycosides, fluoroquinolones, and β-lactams (penicillins and cephalosporins)[2]. The most common mechanism of carbapenem resistance in Acinetobacter species is the production of carbapenem hydrolyzing class D β-lactamases (CHDLs) [3]. However, the vast of OXA carbapenemases (class D) have been discovered in *A. baumannii*, In addition, the insertion sequence ISAba1 has been found in many isolates of *A. baumannii* located upstream of the bla(*oxa-23*, *oxa-51* and *oxa-58*) carbapenemase genes [4]. Ambler class D (oxacillinases) are sources of multidrug resistance in *A. baumannii* [5]. This study aim to determine the antimicrobial susceptibility patterns of *A. baumannii* isolates from different clinical sources and detection the presence of class D β-lactamases genes bla($_{oxa-51-Like}$, $_{oxa-143-Like}$ and $_{oxa-58-Like}$)genes and insertion sequence ISAba1 in *A. baumannii*.

Materials and methods

Sample collection

A total of 100 clinical specimens of urine, sputum, (wounds, burns and ear) swabs were collected from different Hospitals of Baghdad during the period from December 2013 to May 2014.

Antimicrobial susceptibility Test

According to Kirby-Bauer, method was dependent in Antimicrobials susceptibility test for 16 different antimicrobial agents [6].

DNA Extraction (salting out method)

The method was used in this study described by Pospiech and Neumann [7] to isolate total DNA.

Polymerase Chain Reaction (PCR) Techniques [8]

Series of PCR reactions were performed to detect the three groups of carbapenem hydrolysing class D β–lactamases genes (CHDLs) (Oxacillinases) including bla$_{OXA51like}$, bla$_{OXA58\ like}$ and bla$_{OXA143\ like\ genes}$ and mobile genetic elements such as ISAba1, with specific primers for each gene as showed in table 1.

The program that used in the thermocyler PCR was carried out in which annealing at 58°C for 45sec.

Table 1-The primers used

No.	Target gene	Primer	Oligonucleotide sequence (5'-3')	Product size(bp)
1-	bla$_{oxa51-like}$gene	OXA51-F	5'-TCGACCGAGTATGTACCTGC-3'	501bp
		OXA51-R	5-'TTGAGGCTGAACAACCCATC-3'	
2-	bla$_{oxa58-like}$gene	OXA58-F	5′AAGTATTGGGGCTTG TGCTG-3′	825bp
		OXA58-R	5′CCCCTCTGCGCT CTA CATAC-3′	
3-	bla$_{oxa143\ like}$ gene	OXA143-F	5′-AGTTAACTTTCAATAATTG-3′	908bp
		OXA143-R	5′-TTGGAAAATTATATAATCCC-3′	
4-	Insertion sequence (ISAba1)	ISAba1-F	5′TGGCCTATTTGGAAAGGTTG-3′	291bp
		ISAba1-R	5'-ATTGCCCGTTGAATATCTGC-3'	

Results and discussion

Identification of *Acinetobacter baumannii*

Fifteen isolates (15%) were identified to *A.baumannii* according to cultural characteristics, microscopic examination, and biochemical test and confirmed the identification by using VITEK2 system from one hundred samples.

Antimicrobial Susceptibility test

The Susceptibility of 15 isolates of *A.baumannii* towards 16 different antibiotics were examined by using Kirby-Bauer method. The results showed high level resistance of *A.baumannii* isolates to most antimicrobial agents used in this study, all isolates were resistant to penicillin group included Cloxacillin, Oxacillin, Carbencillin, Methicillin 100% while, Amoxicillin and Piperacillin were 93.33 %. In addition, *A.baumannii* isolates showed the high resistant against third generation of cephalosporin group including Cefotaxime, Cefixime and Ceftazidime in percentage (100,93.33 and 80)% respectively, the result agree with local study in Baghdad city who is found *A.baumannii* isolates were resistant 100% for Cefotaxime and 89.57% for Ceftazidime[9].

Present study showed resistant percentage 87% to Carbapenem such as Imipenem and Meropenem, and 73% resistant to Aminoglycoside such as Amikacin, Besides the resistant to (Amoxicillin+Clavulanic acid, Ciprofloxacin, Tetracyclin , Trimethoprim+sulfamethoxazole) in percentage (87, 73, 60 , 87)% respectively. The local study in Baghdad city found that various levels of resistant to several antimicrobial agents, that in agreement with results of the current study included carbencillin(100%), Cefotaxime(100%), Cefixime100%.while other results were disagreed with present study included Amikacin 50%, Ceftazidime 100%, Ciprofloxacin 63.63%, Imipenem 40%, Meropenem 50% and Tetracyclin 77.27%[10]. These differences in the results may be attributed to excessive use antimicrobial agents in the hospitals in the last few years. Probability, the differences in the results may be retained to increasing the spread of multidrug resistant mechanisms by genetic elements (mobile elements).

In previous study point out Acinetobacter *spp*. collected between 1994 and 1995 in five European countries from ICUs showed susceptibilities to ceftazidime of 82%in Belgium, 19%in Portugal, 30%in France, 24%in Spain and 100%in Sweden, while, Susceptibility to imipenem was 88%in Belgium, 91%in France, 84%in Spain,95%in Portugal and 81%in Sweden[11,12] .the susceptibility results by these previous study of Imipenem were compatible with the results of present study whereas Susceptibility of ceftazidime was incompatibility due to geographic site and β-Lactamase production. Meanwhile, Spanish study has also documented significant levels of resistance, ceftazidime resistance increased from 57.4% in1991 to 86.8%in 1996, while imipenem resistance increased from 1.3%to 80.0% either Ciprofloxacin resistant increased from 54.4% to 90.4% also Amikacin 21% to 83.7%, Ceftazidime 57.4% to 86.8% and Trimethoprim-sulfamethxazole 41.1% to 88.9%[13].

On the other hand, the resistance of *A. baumannii* to antimicrobial agents is mediated by all of the major resistance mechanisms known to occur in bacteria including degradation enzymes against ß-lactams, modification enzymes against aminoglycosides, altered binding sites for Quinolones, and a variety of efflux mechanisms and changes in outer membrane proteins have been reported[1].

Molecular study of *Acinetobacter baumannii* genes using PCR Technique

Screening for the presence of ISAba1

The results showed a band of PCR product with 291bp that represented insertion sequence (ISAba1) in 13 isolates of *A.baumannii* resistant to Oxacillin as illustrated in figure 1. The recent study in Egypt revealed that all 40 *A.baumannii* isolates had bla$_{oxa-51}$and ISAba1, The ISAba1 element was found upstream to the genes bla $_{oxa-51}$and bla$_{oxa-23}$ in percentages (85 and 80)% respectively ,The ISAba1 element was not found upstream of bla$_{\text{OXA-51like}}$gene in six isolates(15%)[14]. Other study confirmed the results of present study, in which reported that in all thirty five MDR *A. baumannii* isolates from Korea were amplified bla$_{\text{OXA-51-like}}$ gene; the element ISAba1was identified upstream for 21 isolates[15]. In particular, the region upstream of OXA-type class D carbapenemase in *A.baumannii* frequently includes the insertion sequence (IS) ISAba1, which can regulate the expression OXA-type carbapenemase genes, an IS is a mobile genetic element known to influence the developmental pattern of bacterial genomes, additionally, the IS elements may cause DNA insertion/deletion, chromosomal rearrangement, and modulation of neighboring gene expression, thereby influencing the bacterial phenotype [15,16].

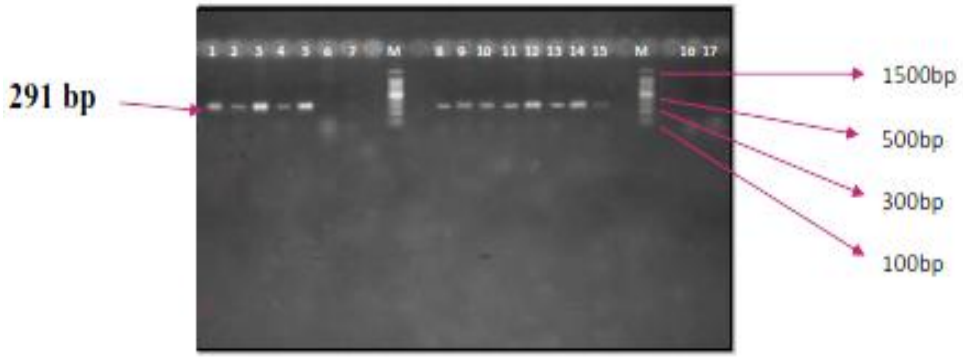

Figure 1-Agarose gel electrophoresis of PCR product using specific primer for detected ISAba1 element in *A.baumannii*, Electrophoresis was performed on 2 % agarose gel and run with a 5volt/cm for 1:30 hr. stained with Ethidium Bromide and visualized on a UV transiluminator documentation system. M: ladder 100bp. Lines (1-15) meaning (Ab1-Ab15). line16 :(control negative, PCR reaction without DNA).Line17 :(control negative, susceptibility isolate).

Detection of Carbapenem Resistance Genes (Oxacillinases) using Polymerase Chain Reaction (PCR) Technique

The isolates were screened by PCR technique for the bla$_{OXA\text{-}51like}$, bla $_{OXA\ 58\ like}$ and bla $_{OXA\ 143\ like}$ genes using specific primers for each one. The results showed that twelve isolates out of fifteen isolates of *A. baumannii* carried the bla $_{\text{OXA-51like}}$ gene resistance to carbapenems (Oxacillin) as illustrated in figure (2); on the other hand, the results of present study showed that all carbapenem resistant *A. baumannii*(CRAB) isolates were lack for bla $_{OXA\text{-}58\text{-}like}$ and bla $_{OXA\ 143\ like}$ genes. These results were compared with negative control isolates sensitive to Oxacillin.

A previous study in Greece by Pournaras *et al*. [17] referred that 15 clinical isolates of *A.baumannii* carrying the bla $_{OXA\text{-}51\ like}$ gene from 17 isolates using PCR technique, but when qRT-PCR is used; it showed that bla$_{OXA\text{-}51\text{-}like}$ genes were expressed in 12 isolates. On the other hand, study by Netsvyetayeva *et al*. (2011) noted 15 among 16 analyzed *A. baumannii* isolates displayed bla $_{\text{OXA-51like}}$ gene [18]. The negative amplification of this gene may due to either point mutation or the isolates were not harboring this gene. The study done by Liang *et al.*,(2012) referred that 115 clinical isolates of *A.baumannii* collected from 10 general hospitals in Changsha, were positive for the amplification of the bla $_{OXA\text{-}51\text{-}like}$ genes, but negative for the amplification of the bla $_{OXA\text{-}58like}$ genes[19].

Martins *et al*. [20] reported that Fifty-three clinical *A.baumannii* isolates were identified in south of brazil, They mentioned that all carbapenem resistant *A. baumannii* (CRAB) isolates carried bla$_{\text{OXA-51-like}}$ gene, but negative for bla $_{OXA\text{-}58\text{-}like}$ genes. A study by Mostachio *et al.*,[21] found that 76% isolates harbored the bla $_{OXA\text{-}143\ like}$ gene in Brazil. The recent study in Egypt by Al-Agamy *et al*.[14] revealed that 40 isolates of *A. baumannii* had bla$_{OXA\text{-}51like}$ gene, but 2 isolates among the 40 *A. baumannii* isolates collected had bla $_{OXA\text{-}58\text{-}like}$ genes. As well as, recent study in Lebanon reported that all *A.baumannii* isolates showed negative result for bla $_{OXA\ 143\ like}$ gene [22]. The resistance of *A. baumannii* (not harboring carbapenem resistance genes) to antimicrobial agents occurs by other carbapenem resistant genes, altered binding sites for Quinolones, a variety of efflux mechanisms and changes in outer membrane proteins [23].

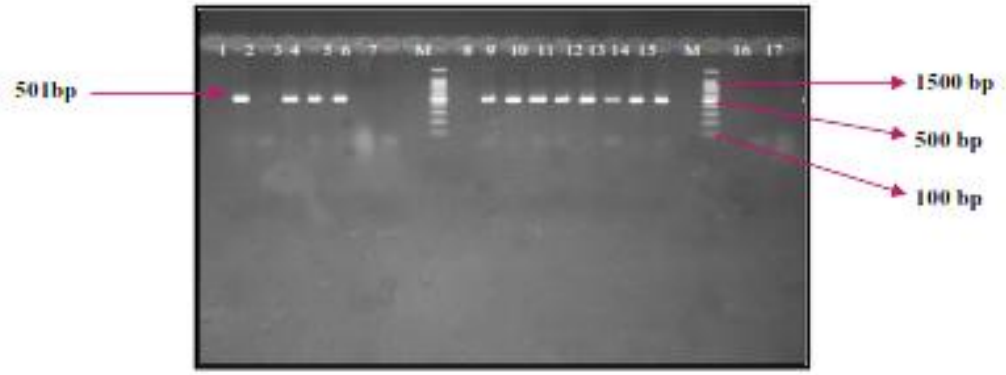

Figure 2-Agarose gel electrophoresis for PCR product for detected bla$_{OXA51like}$gene. Electrophoresis was performed on 2 % agarose gel and run with a 5volt/cm for 1.30 hr. stained with Ethidium Bromide and visualized on a UV transiluminator documentation system.M: 100bp ladder. Line 16 :(control negative, PCR reaction without DNA).Line 17 :(control negative, sensitive isolate). Line (1-15) meaning Ab1-Ab15.

Reference:

1. Fishbain, J.and Peleg, A.Y.‹**2010**›. Treatment of *Acinetobacter* Infections, *Clin. Infect. Disea*. 51(1):79–84.
2. Siroy, A.; Molle, V.; Lemaitre-Guillier, C.; Vallenet, D.; Pestel-Caron, M.; Cozzone, A.J.; Jouenne, T. and De, E.‹**2005**›. Channel Formation by CarO, the Carbapenem Resistance-Associated Outer Membrane Protein of *Acinetobacter baumannii*. *Antimicrob. Agents and Chemoth.* 49(12), pp: 4883-4876.
3. Lee, Y.; Kuo, S.; Chiang, M.; Yang, S.; Chen, C.; Chen, T., and Fung, C.‹**2011**›. Emergence of Carbapenem-Resistant Non-baumannii Species of Acinetobacter Harboring a bla $_{OXA-51-Like}$ Gene That Is Intrinsic to *A. baumannii*. *Antimicrob. Agents and Chemoth*. 56(2), pp: 1124 –1127.
4. Martinez, P. and Mattar, S.‹**2012**›. Imipenem-resistant *Acinetobacter baumannii* carrying the ISAba1-bla oxa-23, 51 and ISAba1-bla adc-7 genes in monteria, Colombia. Brazilian *J. Microbiol*. 43(4), pp: 1274-1280.
5. Nagano, N.; Nagano, Y.;Cordevant, C ;Shibata, N. and Arakawa, Y. ‹**2004**›. Nosocomial Transmission of CTX-M-2 β -Lactamase-Producing *Acinetobacter baumannii* in a Neurosurgery Ward. *J. Clin. Microbiol.* 42 (9), pp: 3978-3984.
6. CLSI, (Clinical and Laboratory Standards Institute) ‹**2012**›. Performance standard for antimicrobial susceptibility testing; Approved Standard-Eleventh Edition. M02-S11. 32(1).
7. Pospiech, J. and Neuman,T.‹**1995**›.Preperation and analysis of genomic and plasmid DNA (ed Kieser,T),Norwich, U.K.
8. Nowak, P.; Paluchowska, P.and Budak, A. ‹**2012**›. Distribution of blaOXA genes among carbapenem-resistant Acinetobacter baumannii nosocomial strains in Poland. *New Microbiol*. 35, pp: 317-325.
9. AL-Saleem, N.H.H.‹**2013**›. Genotyping relatedness of *Acinetobacter baumannii* isolated from Medical City/Baghdad. Ph.D. Thesis. Biology department.College of Sciences. Baghdad University.
10. Al-Mash'hadani, E. I. J. ‹**2010**›. Study The activity of Bacteriocin produced from *Lactobacillus plantarum* on virulence factors of *Acinetobacter baumannii*. M.Sc. thesis. Biology department. College of Science. AL-Mustansiriyah University.
11. Glupczynski, Y.; Delmee, M.; Goossens, H. and Struelens, M.A.‹ **1998**›. Multicentre survey of antimicrobial resistance in Gram negative isolates from Belgian intensive care units in 1994–1995. Belgian Multicenter ICU Study Group.*Acta. Clinica.* Belgica. (53), pp: 28–38.
12. Hanberger, H.; Garcia-Rodriguez, J.A.; Gobernado, M.; Goossens, H.; Nilsson, L.E. and Struelens, M.J.‹ **1999**›. Antibiotic susceptibility among aerobic Gram-negative bacilli in intensive care units in 5 European countries. French and Portuguese ICU Study Groups. *J. of the American Med. Assoc*. (281), pp: 67–71.

13. Ruiz, J.; Nunez, M.L.; Perez, J.; Simarro, E.; Martinez-Campos, L.and Gomez, J.‹ **1999**›. Evolution of resistance among clinical isolates of Acinetobacter over a 6-year period. *European J.of clin.Microbial.*& *infect. Dis*.(18), pp: 292–295.

14. AL-Agamy, M.H.; Khalafc, N.G.; Tawfick, M.M.; Shibla, A.S. and Kholy, A.E.‹**2014**›. Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt, *Intern. J. of Infect. Dis*. 22, pp: 49–54.

15. Koo, S.H.; Kwon, K.C.; Cho, H.H. and Sung, J.Y.**.,**‹**2010**›. Genetic Basis of Multidrug-resistant Acinetobacter *baumannii* Clinical Isolates from Three University Hospitals in Chungcheong Province, Korea. *The Korean J. of Labora. Medic*. 30:498-506.

16. Gur, D.; Korten, V.; Unal, S.; Deshpande, L.M.and Castanheira, M.‹**2008**›. Increasing carbapenem resistance due to the clonal dissemination of oxacillinase (OXA-23 and OXA-58)-producing *Acinetobacter baumannii*: report from the Turkish SENTRY Program sites. *J. of Medical Microbiol.* 57:1529–1532.

17. Pournaras, S.; Markogiannakis, A.; Ikonomidis, A.; Kondyli, L.; Bethimouti, K.; Maniatis, A.N.; Legakis, N.J.and Tsakris, A.‹**2006**›. Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing OXA-58 carbapenemase in an intensive care unit. *J. of Antimi. Chemoth*. 57, pp: 557–561.

18. Netsvyetayeva, I.; Sikora, M.; Golas, M.; Swoboda-Kopec, E.; Walter de Walthoffen, S.; Dembicka, O.; Fraczek, M.; Mlynarczyk, A.; Pacholczyk, .M; Chmura, A. and Mlynarczyk, G.‹**2011**›. *Acinetobacter baumannii* Multidrug-Resistant Strain Occurrence in Liver Recipients With Reference to Other High-Risk Groups. Transplantation Proceedings.43: 3116 –3120.

19. Liang, W.; Zou, M.; Wu, J.; Wu, G.; Li, J.; Dou, Q. and Liu, W. ‹**2012**›. Molecular epidemiological characteristics of clinically isolated carbapenem resistant *Acinetobacter baumannii* in Changsha. *J. of Cent. South Unive- Med Sci*. 37(5): 521-526.

20. Martins, A. F.; Kuchenbecker, R.; Sukiennik, T.; Boff, R.; Lutz, L. And Barth, A.L.‹**2009**›. Carbapenem-Resistant *Acinetobacter baumannii* Producing the OXA-23 Enzyme: *Dissem. In Southern Brazil, Infect*. 37, pp: 474-476.

21. Mostachio, A.K.; Levin, A.S.;Rizek, C.; Rossi, F.; Zerbini, J.‹**2012**›. High prevalence of OXA-143 and alteration of outer membrane proteinsin carbapenem-resistant Acinetobacter *spp*. isolates in Brazil. *Intern. J. Antimicrob. Agent*. 39(5): 396-401.

22. Rafei, R.; Dabboussi, F.; Hamze, M.; Eveillard, M.; Lemarie, C.; Mallat, H.; Rolain, J.; Joly-Guillou,M.; Kemp, M.‹**2014**›. First report of blaNDM-1-producing *Acinetobacter baumannii* isolated in Lebanon from civilians wounded during the Syrian war. *Intern. J. of Infect. Dis*. 21, pp: 21-23.

23. Fishbain, J.and Peleg, A.Y.‹**2010**›. Treatment of *Acinetobacter* Infections. *Clin*. *Infect*. *Dis*. 51(1), pp: 79–84.