Diyala Journal for Pure Science

Kps M Detection in Acinetobacter Baumannii Isolated from Different Clinical Cases in Baghdad City

Rana Mujahid Abdullah and Rasha Zaid Tariq Ahmed

Department of Biology – College of Education for Pure Science – Ibn Al Haitham – University of Baghdad

dr.rana_alshwaikh@yahoo.com

Received: 4 December 2019 **Accepted: 31 December 2019**

DOI: https://dx.doi.org/10.24237/djps.16.02.522B

Abstract

Forty isolates of Acinetobacter baumannii were obtained from 200 samples. The isolates were collected from different cases including: - wounds, burns, stool, urine, sputum and blood samples. For the period between 1/9/2016 to 30/11/2016. from several hospitals in Baghdad city (Central Children's Hospital, Al Karama Hospital, Karkh General Hospital, Al-Ameen Medical City Hospital, Educational Labs, Baghdad Teaching Hospital, Child Protection Hospital, Burns and Wounds Hospital). Genotypic detection for virulence genes (*kpsM*) of *Acinetobacter baumannii*, the result revealed that the *kpsM* gene was present in 6 isolates (15%) of *A. baumannii* possessed the *kpsM* gene. The resulting packets with a molecular weight were 272 bp. Sequential analysis detection of *kpsM* showed that nine silent mutations that did not effect of the amino acid translation. While two mutations effected in the translation of amino acids as there was a change in path of translation of the protein, where the conversion of the amino acid (Lysine) to the amino acid (Asparagine) and converted the amino acid (Isoleucine) to the amino acid (Valine).

Keywords: *Acinetobacter baumannii*, *kpsM gene*, sequencing, mutation.

Diyala Journal for Pure Science

Kps M Detection in Acinetobacter Baumannii Isolated from Different Clinical Cases in Baghdad City

Rana Mujahid Abdullah and Rasha Zaid Tariq Ahmed

تحديد لجين *kpsM* في بكتريا Acinetobacter baumannii المعزولة من حالات سريرية مختلفة في مدينة بغداد

رنا مجاهد عبدالله و رشا زياد طارق احمد

قسم علوم الحياة – كلية التربية للعلوم الصرفة ابن الهيثم – جامعة بغداد

الخلاصة

تم الحصول على 40 عزلة لبكتريا *Acinetobacter baumannii* من اصل 200 عينة، وقد تم جمع العزلات من حالات مرضية مختلفة شملت كل من الجروح والحروق والخروج والادرار ومن عينات القشع وعينات الدم. للفترة من 2016/9/1 إلى 2016/11/30. من عدة مستشفيات في مدينة بغداد (المستشفى المركزي للأطفال، مستشفى الكرامة، مستشفى الكرخ العام، مستشفى مدينة الأمين الطبية، المختبرات التعليمية، مستشفى بغداد التعليمي، مستشفى حماية الطفل، مستشفى الحروق والجروح). الكشف عن جين (*kpsM*) لـ Acinetobacter baumannii المسؤول عن تكوين الكبسولة اظهرت النتائج ان جين *kpsM* كان موجودًا في 6 عزلات (15٪) من بكتريا *A. baumannii.* كانت الحزم الناتجة ذات الوزن الجزيئي 272 زوج قاعده. أظهرت نتائج الكشف عن التحليل التتابعي لجين *kpsM* ان هناك تسعة طفرات صامتة لم تؤثر على ترجمة الأحماض الأمينية. بينما اظهرت النتائج ان طفرتين كان لها تاثير في ترجمة الأحماض الأمينية حيث كان هناك تغيير في مسار ترجمة البروتين، اذ تم تحويل الحمض الأميني (لايسين) إلى الحمض الأميني (اسبراجين) وتحويل الحمض الأميني (ايزوليوسين) إلى الحمض الأميني (فالين).

الكلمات المفتاحية : *Acinetobacter baumannii* ، جين *kpsM*، التحليل التتابعي، الطفرات.

Introduction

The capsule is one of the most important virulence factors possessed by *A. baumannii,* which works to protect the bacterial cell from dehydration, phagocytosis, killing that mediates the complement and resistance to antibiotics and works to make cells adherent and this explains the ability of bacteria to survive in healthy environments such as medical devices or surface surfaces as in the case of furniture and bed in a hospital environments [1-3].

The capsule is one of the most important non-ciliary adhesion factors in bacteria, has an important role on adhesion of bacteria on the catheter tubes and formation of biofilms membrane in the environment of hospitals. The presence of capsule with cilia plays an important role in the adhesion of *Acinetobacter* to epithelial cells, mucous membranes of the urinary tract, respiratory tract, intestinal and genital infections of humans [4]. The different of capsule in terms and its components but also in the way of its binding to sugars. Several studies indicated to the relationship between the capsule and the pathogenicity of bacteria [5- 7]. The chemical composition of the *A. baumannii* capsule has high molecular weight polymers of single units of monosaccharides. [8]. The aims of this study to detection of *kpsM* gene and sequencing analysis of this gene and study mutations and their effect on protein translation.

Material and Methods

Collection of samples

Two hundred samples have collected from different sources of infection for the period between 1/9/2016 to 30/11/2016.

Identification of bacteria

The samples were inoculated on MacConkey agar, blood agar then identified by performing biochemical tests, further identification by using API20E system and VITEK-2 system [9].

Isolates of DNA

DNA kit (Geneaid Biotech kit system, UK) was used to extract the DNA of bacterial isolates according to the manufacturer's instructions.

Primers solutions

The solutions were prepared of the Stock according to the instructions of the manufacturer Alpha DNA (Canada) table 1 using deionized nuclease free water to obtain a concentration of

100 picomol / microliter. The solution of each initiator was presented separately at 10 picomol / Microliter by taking 10 microliters of each stock solution and added to 90 microliters of distilled water and mixed well with Vortex mixture, kept with stock solutions at -20°C and mixed the initiator solution after removed it from ice (manufacturer Alpha DNA).

Table 1: The sequence of the primer that used in the study

Genes	Sequencing Primer sequence (5'——3')	(bp)Product	Reference
kpsM	GCGCATTTGCTGATACTGTTG CATCCAGACGATAAGCATGAGCA	272	Johnson and Stell (2000) [10]

The PCR polymerase reaction mixture, *kpsM* gene was prepared as shown in table 2.

Table 2: PCR mixture of the genes using in this study

No.	PCR mixture	Size (µl)
1	F-primer	1
2	R-primer	1
3	Template DNA	2
4	Nuclease Deionized nuclease-free water	6
5	GO Taq green master mix	10
	Total	20

The contents of the PCR tubes were mixed well using the Vortex and then placed in a PCR thermal cycler.

Table 3: Optimal conditions for PCR reaction to investigate *kpsM* gene

Step	Program	
1	Only one cycle for 6 minutes at a temperature of 94 ° C for the primary DNA denaturation.	
2	34 cycle included:	
	A	50 sec at 94 ° C for DNA template denaturation.
	B	70 sec at 55 °C for the primers to bind to DNA template annealing at 58 °C for 70 sec.
	C	55 sec at 72 ° C for the associated primers to be elongated.
3	Only one cycle for10-minute at 72 ° C for the final elongation of the double DNA strip.	

Separation of DNA bands

PCR products were separated on a 2% agarose with 5µl Ethidium bromide, at 100 vol. for 80 min. The DNA bands were visualized and photographed under UV light [11].

Result and discussion

The isolates had identified by culturing on MacConkey, blood agar then identified by performing biochemical tests including (oxidase test, catalase test), further identification by using API20E system and VITEK-2 system. After identification 40 isolates confirmed to be *Acinetobacter baumannii* included 9 isolates from blood, 1 isolate from urine, 4 isolates from wound infections and sputum each of them, 8 isolates from burns and 14 isolates from stool sample.

A. baumanni, kpsM genes was detected using PCR technique. The results showed that the selected isolates possessed these genes in different proportions. The results showed that 6 isolates (15%) of *A. baumannii* possessed the *kpsM* gene. The resulting packets with a molecular weight were 272 bp as shown in figure 1.

The result was closely correlated with Momtaz *et al.* [12] findings they have found 14.04% of *A. baumannii* isolates possessed *kpsM* gene whereas Joly-Guillou [13] did not find the genes in *A. baumannii*.

The results of Al-Warid [14] showed that the proportion of *A. baumannii kpsM* gene Reach to 54%, and *kpsM* gene encoded for the production of the portfolio in the bacteria and an important virulence factor as it works to form biofilm, and non-ciliary adhesion factors in bacteria and protects the bacterial cell from dehydration, phagocytosis and components of the Complementary System Components [3].

Diyala Journal for Pure Science

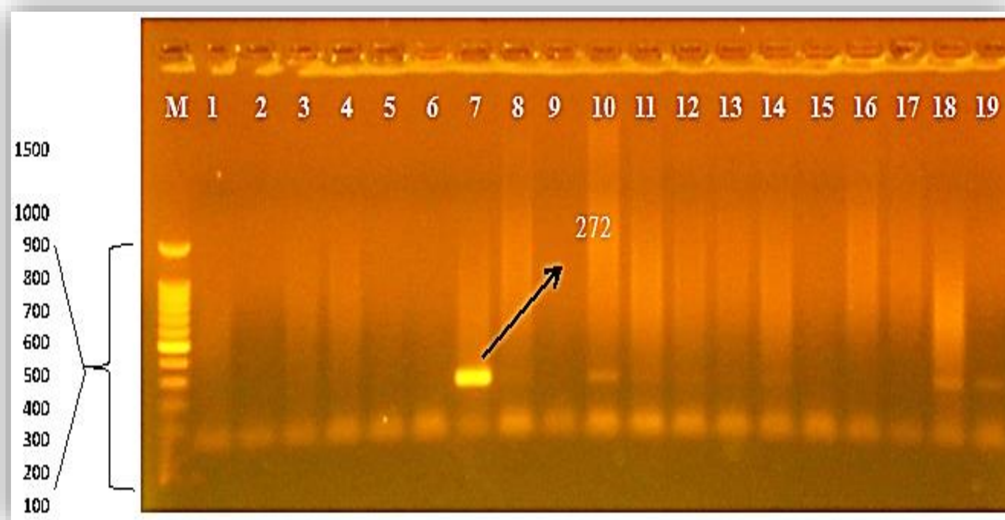

Figure 1: Agarose gel (2%) electrophoresis of the PCR product of the *kpsM* gene (272bp) of *A. baumannii* isolates on 100 volts for 80 min. Line M: DNA marker (100-1500bp Ladder, Promega, USA); Lanes (7,10,18,19) *A. baumannii* PCR positive isolate.

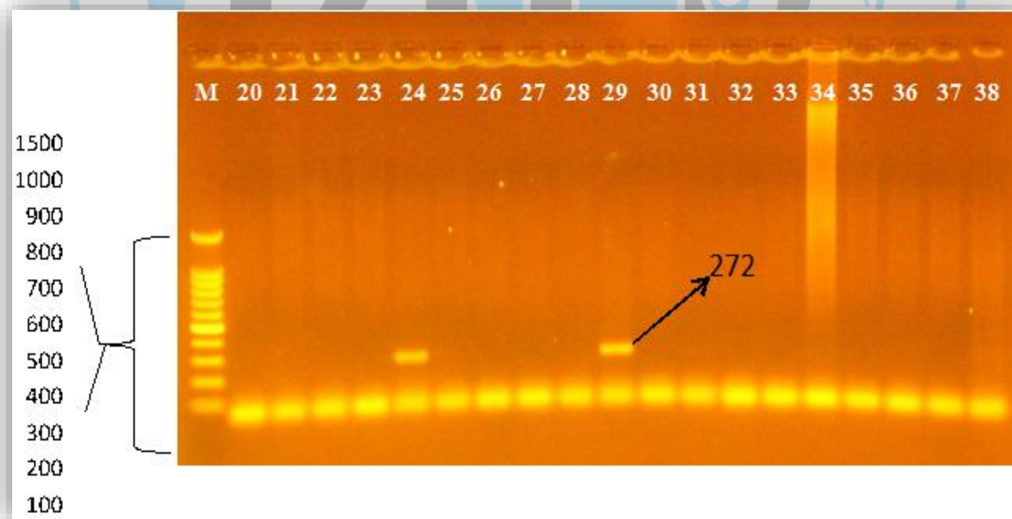

Figure 2 : Agarose gel (2%) electrophoresis of the PCR product of the *kpsM* gene (272bp) of *A. baumannii* isolates on 100 volts for 80 min. Line M: DNA marker (100-1500bp Ladder, Promega, USA); Lanes (24, 29) *A. baumannii* PCR positive isolate.

The DNA sequence of *kpsM* was detection in *A. baumannii* in this study, and the results showed that there were eleven mutations of genotype substitution and addition in the isolates A5 and A10 (isolated from burns and stool respectively). The result confirmed that nitrogen base Guanine was replaced by Adenine in Position 66 at the Subject 3598980, and replaced the Thymine nitrogen base with Cytosine nitrogen base Position 114 at the Subject 3598954 and Position 120 at the Subject 3598960, respectively. Adenine was replaced by Thymine at the position of 126 at the Subject 3598916 and Adenine replaced with the nitrogenous base Guanine at position 133 at the Subject 3598915. The thymine base was replaced by adenine in Position 135 at Subject 3598917 and Cytosine was replaced by the thymine in Position 153 at Subject 3598896. Adenine was replaced by Guanine in Position 159 at Subject 3598892, and \Position 204 at Subject 3598852. Cytosine was replaced by Thymine in Position 211 at Subject 3598839 and Thymine was replaced with Adenine at Position 246 at Subject 3598824, as shown in table 4 and figure 3.

The results of the translation of the amino acid coding gene (*kpsM*) with the results of the translation of the original amino acid was found that there is an effect of mutations in the translation of amino acids as there was a change in the path of translation of the protein, where the conversion of the amino acid (Lysine) to the amino acid (Asparagine) and converted the amino acid Isoleucine to the amino acid Valine.

The mutations in *kpsM* are functional nsSNPs, the changes in amino acids of this gene are novel and not detected in another study.

Table 4: Changes in nitrogen bases and their effect on the translation of *kpsM* gene for the isolates A5 and A10 of *A. baumannii.*

No.	Nitrogenous base	Changes in nitrogen bases	Position	Subject	Amino acid	Changes in amino acid
1	Guanine	Adenine	66	3598980	Phenylalanine	Phenylalanine
2	Thymine	Cytosine	114	3598954	Glycine	Glycine
3	Thymine	Cytosine	120	3598960	Isoleucine	Isoleucine
4	Adenine	Thymine	126	3598916	Lysine	Asparagine
5	Adenine	Guanine	133	3598915	Isoleucine	Valine

Diyala Journal for Pure Science

Kps M **Detection in Acinetobacter Baumannii Isolated from Different Clinical Cases in Baghdad City**

Rana Mujahid Abdullah and Rasha Zaid Tariq Ahmed

6	Thymine	Adenine	135	3598917	Alanine	Alanine
7	Cytosine	Thymine	153	3598896	Aspartic	Aspartic
8	Adenine	Guanine	159	3598892	Alanine	Alanine
9	Adenine	Guanine	204	3598852	Lysine	Lysine
10	Cytosine	Thymine	211	3598839	Lysine	Lysine
11	Thymine	Adenine	246	3598824	Serine	Serine

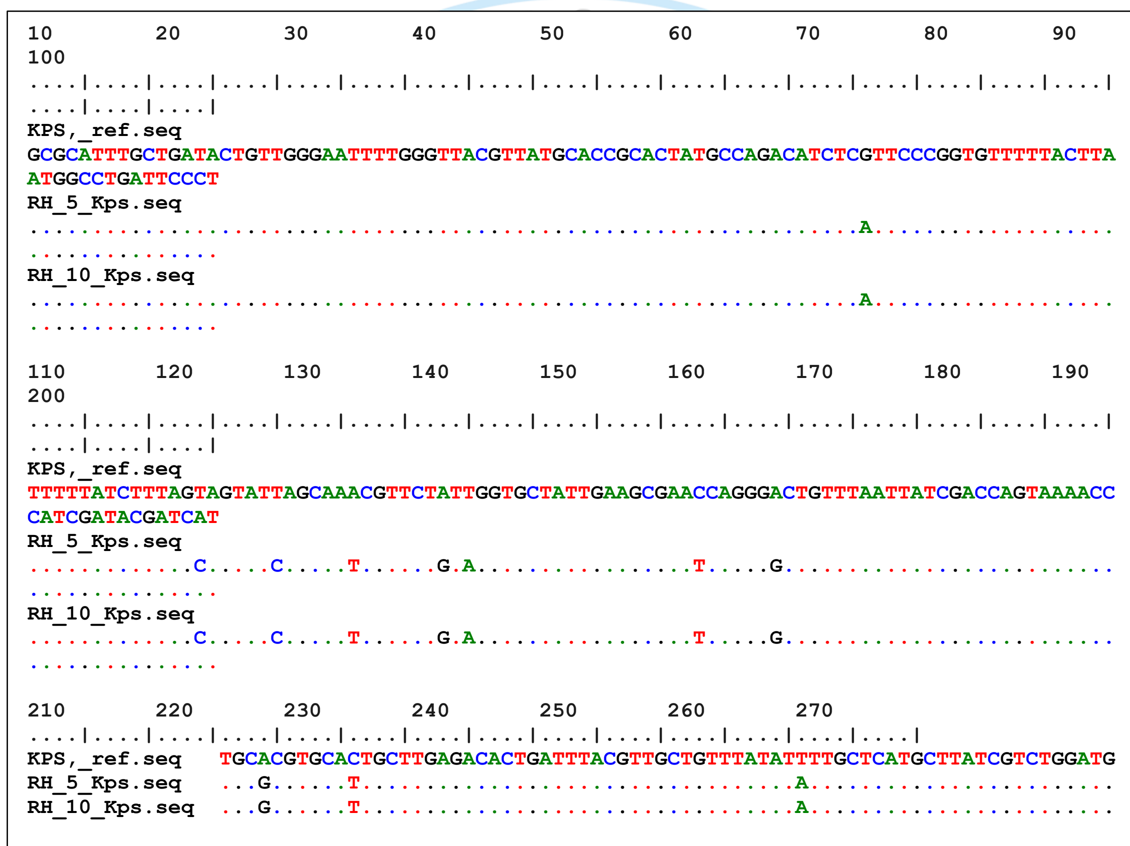

Figure 3: Analysis of multiple gene sequences of *kpsM* reference with two changes to A5 and A10 isolates of *A. baumannii* using the BioEdit Sequence Alignment Editor Software.

The ***kpsM*** gene has been registered at National Center for Biotechnology Information (NCBI) at the following link: https://www.ncb.nlm.nih.gov/nuccore/LC218743

Conclusions

kpsM Sequencing analysis showed two mutations led to a change in amino acid and protein translation. While other mutation not effect on protein translation.

References

1. Y. L. Zhang, Y. L. Lau, E. Arakawa, K. Y. Leung, Microbiology, 149 (4), 1051-1060 (2003).

2. A. P. Tomaras, C. W. Dorsey, R. E. Edelmann, L. A. Actis, Microbiology, 149 (12), 3473-3484 (2003).

3. G. Braun, M. Vidotto, Memórias do Instituto Oswaldo Cruz, Rio de Janeiro, 99 (8), 839-844 (2004).

4. R. M. Hashem. Production and purification of alkene from Acinetobacter baumannii isolated from sputum and its effect on some microorganisms outside the body, Master Thesis, College of Science, Al-Mustansiriya University, pp. 145 (2014). (Reference in Arabic).

5. H. E. Smith, M. Damman, J. van der Velde, F. Wagenaar, H. J. Wisselink, N. Stockhofe - Zurwieden, M. A. Smits, Infection Immune, 67(4), 1750-1756 (1999).

6. Y. Doi, K. Yokoyama, K. Yamane, J. Wachino, N. Shibata, T. Yagi, T. Yagi, K. Shibayama, H. Kato, Y. Arakawa. Antimicrobial Agents and Chemotherapy, 48 (2), 491-496 (2004).

7. E. I. J. Al-Mashhadani, Study of the efficacy of the Bacteriocin produced by Lactobacillus plantarum in the virulence factors of Acinetobacter baumannii. Master Thesis, College of Science, Al-Mustansiriya University, pp. 120 (2010). (Reference in Arabic).

8. I. S. Roberts, Annual Reviews of Microbiology, 50, 285 - 315 (1996).

9. E J, Baron, S. M. Finegold, I. L. R. Peterson, Bailey Scott's, Diagnostic Microbiology, 13th ed. (Mosby Company, Missouri, 2014).

10. J. R. Johnson, A. L. Stell. Journal of Infection Diseases, 181, 261-272 (2000).

11. J. Sambrook, D. W. Rusell. Molecular cloning a laboratory manual, (Cold spring Harbor, NY. Cold spring Harbor Laboratory press, 2001).
12. H. Momtaz, S. Seifati, M. Tavakol, International Journal of Medical Laboratory,2 (2), 87-97 (2015).
13. M. L. Joly-Guillou, Clinical of Microbiology Infection, 11 (11), 868-873 (2005).
14. R. Al-Warid, Immunological and Molecular Study on Acinetobacter baumannii Isolated from Clinical Samples, Ph. D. Thesis, University of Babylon, Iraq (2014).