PalArch's Journal of Archaeology of Egypt / Egyptology

EVALUATE THE RELATION BETWEEN *LUXS* GENE AND THE BIOFILM PRODUCTION BY *KLEBSIELLA PNEUMONIAE*

Adnan Ali Hammad[1], Jaafar Ali Mohammed[2], Sumaya Ayad Abdulrazzaq[3] and Saade Abdalkareem Jasim[3*]

1. Anbar Educational Directorate, Ramadi city, Al-anbar, IRAQ

2.Department of Religious Education and Islamic Studies, The Sunni Endowment Office, Diyala, IRAQ

3.Department of Medical Laboratory Techniques, Al-maarif University College, Ramadi,Al-anbar, IRAQ

***E-mail:** saade.a.j@uoa.edu.iq

Adnan Ali Hammad, Jaafar Ali Mohammed, Sumaya Ayad Abdulrazzaq and Saade Abdalkareem Jasim, Evaluate The Relation Between Luxs Gene and The Biofilm Production By Klebsiella pneumoniae-Palarch's Journal Of Archaeology OfEgypt/Egyptology 17(7),ISSN 1567-214x

Abstract

The quorum sensing system control by the output, secretion and identification of extracellular signal molecules called auto-inducers. The Gram-negative bacterium Klebsiella pneumoniae consider as a multidrug-resistant pathogen that spread globally. Biofilms provide conditions that shield cells from adverse environmental influences, for this reason this study aimed to detect the relation between one of the QS gene (LUX). One hundred tewnty isolates were collected from patients suffered from pneumonia infection but only 22 isolates were K. pneumonia by the API system. Biofilm production were calculate and RNA were extract and then converted to cDNA to perform real time- PCR done for those samples to detect the gene expression. the results showed that only 17 samples possess the LUX gene and The LUX gene does not contain 5 isolates and those five isolates showed a lower biofilm production level. While 17 isolates indicates a higher level of development of biofilm, additionally, a positive and significant correlation were confirm between biofilm production and LUX gene expression. From the above approach results, we can conclude that, LUX gene effect strongly on the production of biofilm and the rate of LUX gene expression positively correlated with biofilm production.

Key words:*Klebsiella pneumonia*, quorum sensing, LUX gene, Biofilm, PCR.

Introduction

Klebsiella pneumoniae possess a number of virulence factors which share with pathogenicity and include capsule antigens, adhesion factors, enterotoxin produce like lipopolysaccharide as well as resistance killer effect for serum and system the

gain on iron (Siderophore) and multi resistance for antibiotics which considered the main reason in spread acquired infections in hospitals[1]. Quorum Sensing (QS) is a cell-to - cell communication system which allows bacteria to control biological functions of response to population density alterations, therefore serving as an adaptation mechanism for the environment[2,3]. The system is regulated by the development, secretion and recognition from extracellular signaling molecules deemed auto-inducers[4]. Two major groups from auto-inducers are described by two systems, Type I QS auto-inducer-1 molecules are N-acyl homoserine lactone (AHL) derivatives ,and Type II QS signaling molecules are defined as auto-inducer-2 (AI-2) [5]. AI-2 is generated by the LuxS enzyme and transforms S ribosylhomocysteine (SRH) to 4,5-dihydroxy-2,3 pentanedione (DPD)[6].The DPD type is Unstable and random cycling to type a furanosyl borate diester (AI-2 molecule). Gram-negative bacterium *K. pneumoniae* has evolved as a multidrug-resistant pathogen that has prevalence worldwide,and is recognized as a cause for invasive blood-borne infections, and also pneumonia and UTI, especially in healthcare settings[7].Biofilms provides arrangements Physically protecting cells of hostile environmental agents, antimicrobials or component from the immune system and causing several chronic infections , especially those associated with indwelling medical devices[8,9].

Materials and methods

Bacterial isolate and growth conditions

Between October 2019 February 2020. One hundred and twenty samples isolated were obtained from patients suffering from pneumonia infection . The study showed22 positive result from the *Klebsiella pneumoniae*, and identified using an API 20E System the procedure was done as recommended by the manufacturer's instruction (BioMérieux, France). It was refreshed in brain-heart infusion (BHI) broth medium at 37ºC for 24 hr.

Biofilm Model

New Colony from*K. pneumoniae* isolates has been utilized to inseminate 5 mL from BHI of the broth medium (Merck Co., Germany),after Incubated period, the number of cells in each culture was quantified and updated to 0.5 McFarland (1.5 × 108 CFU / ml), respectively. Aliquoting 100µl in 96 well, flat bottom, non-tissue culture polystyrene plates (Corning).Media-containing wells alone have been used as negative controls. After incubation at 37 ° C for 24 hours, the

planktonic cells were removed and the wells were washed twice with dH2O. Biofilms were stained with 150µl 0.1 percent (w / v) crystal violet for 15min and wells were rinsed twice with dH2O. Stained biofilms were solubilized with 95 % ethanol and quantified by measuring the OD630 utilizing an Infinite PARA medically reader[10].

RNA extraction

The extraction of RNA was performed utilizing Trizol from all the state's cells in this study according to the protocol provided by the manufacturer of Zymo Quick-RNA Micro-prep Kit. A set of primers were used to amplify the specific gene; forward primer AGGCCAACATATACCACGCC and the reverse primer was TCGCAGGAGAAGATTAGAAAGGA [this study]. And an additional set were use to amplify the reference gene 16SrRNA gene to use it with calculation as a reference gene; F, CCCCGGAAAGGGTCTAACAC and the R, TGAGTGCAAGAGGGGAGAGT . The thermal cycling program was as follow, enzyme activation 95 C for 7 min, followed by 40 cycles of two steps the first one was denaturation 95 C for 20 sec and second step of annealing and florescence screening for 20 sec (55 C) and extension for 20 sec.

Results and Discussion

Out of 120 isolates, only 22 *K. pneumonia* isolates were confirmed by API 20E System. After the Real Time- PCR technique was done to detect the LUX gene expression in those isolates the results showed that only 17 isolates possess the LUX gene while the other five isolates showed no- amplification of the LUX gene. The results in (figure 1) summarize the relation between the presence or absence of LUX gene and the production of biofilm. The group which possessed the LUX showed a higher level of biofilm as they showed a higher level of optical density (0.745), while the group which lack the LUX gene showed lower level of biofilm (0.293).

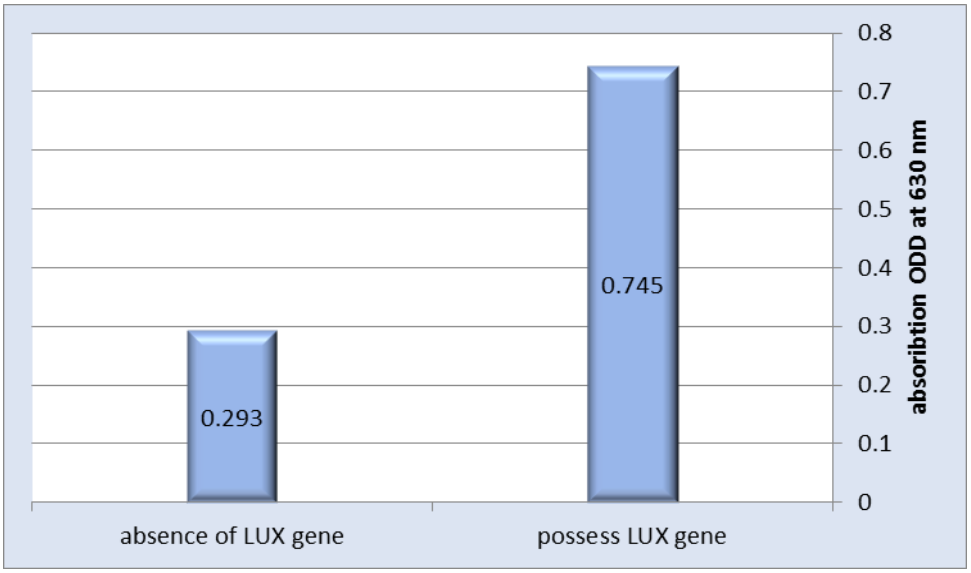

Figure 1 comparision between the biofilm production between the two groups of isolates, theisolates possess LUX gene and the isolates which lack the LUX gene.

The results in (figure 2) showed the amplification curves of real time – PCR for 17 isolates that possess LUX gene while the isolates that lack the gene showed no amplification (straight lines in the figure).

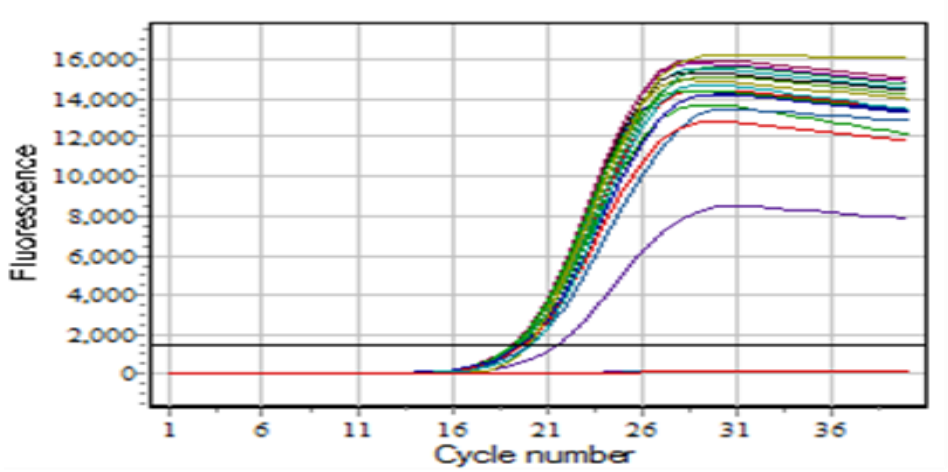

Figure 2 RT-PCR resulted curves of the LUX gene amplification

The correlation between LUX gene expression and biofilm production have been also investigated in this study and the results illustrated in (figure 3) which represents the samples results scattering between both the gene expression and biofilm production. The results showed the slope of the correlation equal to 0.918, and this relation was significant ($p < 0.001$). The calculation of the gene

expression results were calculated according to livack method as shown in table (1). The results of the expression were compared to a control isolate.

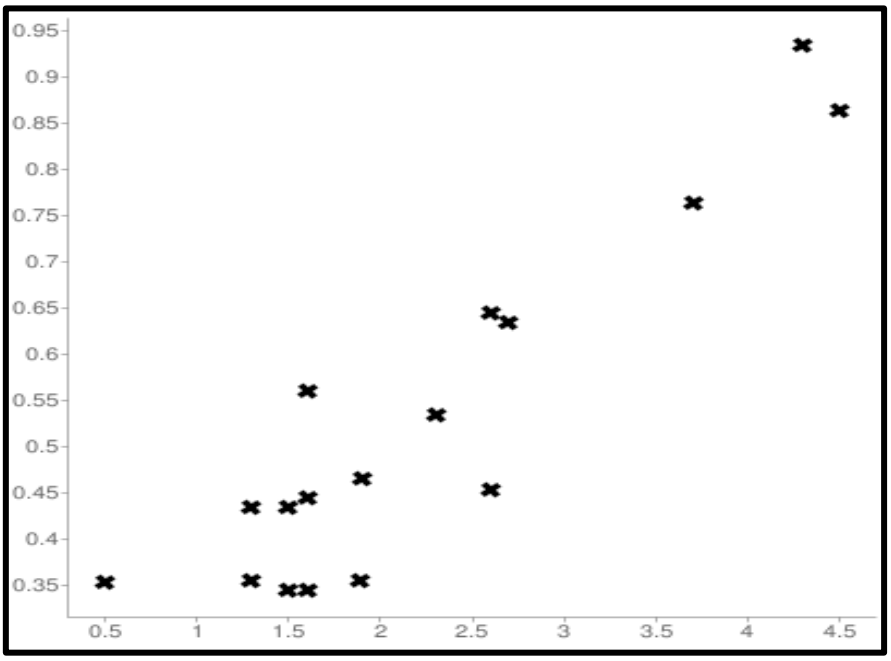

Figure 3 Correlation between the biofilm production on the Y axis and the LUX gene expression on the X axis

- Correlation coefficient (r): 0.9186; calculated by Pearson correlation coefficient, SPSS (version 24).
- p-value < 0.001

Table 1 Calculating of the gene expression

sample No.	16 sRNA	LUX gene	ΔCt	ΔΔCt	folding
1	16.4	18.5	2.1	1.2	0.435275
2	16.4	17	0.6	-0.3	1.231144
3	17.5	18.1	0.6	-0.3	1.231144
4	17.8	18	0.2	-0.7	1.624505
5	16.9	17.1	0.2	-0.7	1.624505
6	17.4	17.5	0.1	-0.8	1.741101
7	17.4	17.2	-0.2	-1.1	2.143547
8	17.8	17.8	0	-0.9	1.866066
9	17.4	17.2	-0.2	-1.1	2.143547
10	17.09	17.4	0.31	-0.59	1.505247
11	17.4	17.2	-0.2	-1.1	2.143547
12	17.8	17.4	-0.4	-1.3	2.462289
13	17.6	17	-0.6	-1.5	2.828427
14	17.8	17.5	-0.3	-1.2	2.297397
15	17.5	16.6	-0.9	-1.8	3.482202

16	17.3	16.1	-1.2	-2.1	4.287094
17	17.4	16.1	-1.3	-2.2	4.594793
Control	15.8	16.7	0.9	-	1

In this study, biofilm formation increased and the gene expression from the luxS gene for *K.pneumoniae* enhanced by quorum sensing, A gene responsible for producing AI-2. AI-2 molecules were previously reported to be involved in *K.pneumoniae* generation biofilms [11]. Likewise, increases in biofilm biomass in the *tqs*A mutant were observed in *E.coli* only when glucose was added to the medium. There is no such regulation in sessile *K.pneumoniae* cells as the associated variations with tqsA mutant biofilms formed in either glucose or glycerol-supplemented medium have been observed. Nevertheless, the finding of a similar phenotype with lux and *tqs*A mutants is puzzling, as in the absence of *tqs*A, AI-2 accumulation within cells should improve the QS-regulated phenotype as observed in *E. coli*[12]. This means that there is no accumulation inside the cytoplasm of "active" AI-2, as opposed to *E.coli*. One hypothesis may be that AI-2 molecules, such as phosphorylates, only become involved after they are imported between transport and phosphorylation via an unknown-paired mechanism. This hypothesis can also explain why the *tqs*A mutant had a substantial increase in lux expression. In particular , increased biofilm biomass and changed architecture have resulted in the deletion of *lsr*CD genes encoding transporters, as with the LuxS-deficient mutant.. [10], A number of studies have identified the importance of exopolysaccharides in the formation of biofilms and their relationship with QS [13,14,15 and 16]. The QS method, the production of biofilms and the growth phase are also likely to be related in *K. pneumoniae* intermediaries, however, remain to be decided. Regulation from the formation of biofilm in *K. pneumoniae* is complex and requires a number from regulatory mechanisms.

Conclusion

We can conclude by this study that LUX gene effect strongly on the production of biofilm which used by the bacteria to colonize and survive. Futuristic studies can use this result to aim the LUX gene in order to minimize bacterial colonization or nearly inhibit.

References

1. Saade Abdulkareem Jasim, Sumaya Ayad Abdulrazzaq, Sarah ibrahim hashoosh, Raed Obaid Saleh. Virulence Factors of *Klebsiella pneumoniae* Isolates from Iraqi Patients.SRP.(2020),11(6):916-921. http://dx.doi. org/10. 5530/srp.2019.2.04.

2. Laganenka L, Colin R, Sourjik V. Chemotaxis towards autoinducer 2 mediates autoaggregation in *Escherichia coli*. Nat Commun 2016;7:12984.

3. Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR. Making 'sense' of metabolism: autoinducer-2, *Lux*S and pathogenic bacteria. Nat Rev Microbiol 2005;3:383–396.

4. Li Z, Nair SK. Quorum sensing: how bacteria can coordinate activity and synchronize their response to external signals? Protein Sci 2012;21:1403–1417.

5. Pereira CS, Thompson JA, Xavier KB. AI-2-mediated signalling in bacteria. FEMS Microbiol Rev 2013;37:156–181

6.Balestrino D, Haagensen JAJ, Rich C, Forestier C. Characterization of type 2 quorum sensing in *Klebsiella pneumoniae* and relationship with biofilm formation. J Bacteriol 2005; 187:2870–2880

7. Calfee DP. Recent advances in the understanding and management of *Klebsiella pneumoniae* . F1000Res 1760; 2017:6 .

8.Stickler DJ. Bacterial biofilms in patients with indwelling urinary catheters. Nat Clin Pract Urol 2008; 5:598–608.

9.Vuotto C, Longo F, Balice MP, Donelli G, Varaldo PE. Antibiotic resistance related to biofilm formation in *Klebsiella pneumoniae* . Pathogens 2014; 3:743–758.

10. Chen, L., Wilksch, J. J., Liu, H., Zhang, X., Torres, V. V., Bi, W., ... & Zhou, T. (2020). Investigation of LuxS-mediated quorum sensing in *Klebsiella pneumoniae*. Journal of medical microbiology, 69(3), 402-413.

11. Balestrino, D., Haagensen, J.A., Rich, C., Forestier, C., 2005. Characterization of type 2 quorum sensing in *Klebsiella pneumoniae* and relationship with biofilm formation. J. Bacteriol. 187, 2870e2880.

12. Herzberg, M., Kaye, I.K., Peti, W., Wood, T.K., 2006. YdgG (*Tqs*A) controls biofilm formation in *Escherichia coli* K-12 through autoinducer 2 transport. J. Bacteriol. 188, 587e598.

13. Balestrino, D., Ghigo, J.M., Charbonnel, N., Haagensen, J.A., Forestier, C., 2008. The characterization of functions involved in the establishment and maturation of *Klebsiella pneumoniae* in vitro biofilm reveals dual roles for surface exopolysaccharides. Environ. Microbiol. 10, 685e701.

14. De Araujo, C., Balestrino, D., Roth, L., Charbonnel, N., & Forestier, C. (2010). Quorum sensing affects biofilm formation through lipopolysaccharide synthesis in *Klebsiella pneumoniae*. Research in microbiology, 161(7), 595-603.

15. Danese, P.N., Pratt, L.A., Kolter, R., 2000. Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. J. Bacteriol. 182, 3593e3596.

16. Prigent-Combaret, C., Prensier, G., Le Thi, T.T., Vidal, O., Lejeune, P., Dorel, C., 2000. Developmental pathway for biofilm formation in curliproducing *Escherichia coli* strains: role of flagella, curli and colanic acid. Environ. Microbiol. 2, 450e464.