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Analysis of virulence genes sequencing of *Serratia marcescens* in iraqi hospitals

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ABSTRACT

Analysis of virulence genes (*PhlA, ShlA, FlhD*) sequencing of *Serratia marcescens* including collection of two hundred twenty samples from sputum & wound infection of the period from April-June in 2021 of the patients in some hospitals in Baghdad – Iraq. These specimens were collected from central hospitals in Iraq. After laboratory diagnosis of these specimens by detecting morphological and biochemical tests on bacteria that were cultured on selective and enriched media, VITEK- 2 compact system. There are 40 bacterial isolates of *Serratia marcescens* from total samples (220) in percentage (18.18%). The genome of these bacteria was extracted to investigate target virulence genes that were amplified by specific forward and specific primers. The product size of virulence genes was *Ph1A* (207 bp), *Sh1A* (217 bp), and *FlhD* virulence gene (307 bp). The results exhibited that these isolates contained these genes at different levels. Sequencing of these genes was carried out and analyzed through BLAST in NCBI and Geneious version -9. The results explained the top identity of sequencing these virulence genes (*PhlA, ShlA, FlhD*) between local Iraqi bacteria. In addition, there are misidentify or dissimilarities in different levels between Iraqi *S. marcescens* and global strain recorded in NCBI. These results consider scientific evidence to find new variations of these virulence genes in Iraqi *S. marcescens in* comparison with the global strain. These new Iraqi bacterial variation sequencing registered in the global database in NCBI under accession numbers including (*Ph1A* virulence gene LC647828.1 & LC647829.1), (*Sh1A* virulence gene LC647830.1 & LC647831.1) & (*F1hD* virulence gene LC647826.1, LC647827.1). The results of analysis sequencing exhibited different percentages in genetic identity distance, which refer to these bacteria new variation in Pathogenicity Island. These results explained the ability of these bacteria to produce different levels of virulence factors that lead to an increase in pathogenicity and spreading of infection.

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Introduction

The bacilli Gram-negative opportunistic pathogen *Serratia marcescens* leads to infection with different diseases considered high morbidity at levels of different ages like neonatal and adult infection in an intensive care unit (1–3). Pathogenicity of these bacteria has a pathogenicity island that includes very important genes encoded for different toxins that cause the destruction of host cells like ShlA, PhlA, FlhD, PigP, and Fimbria for adhesion, lipopolysaccharide (LPS) as well as other important genes that increase from spreading of pathogenicity of these bacteria and the able causative agent for many diseases may rich to bloodstream perhaps lead to death because the understanding of mechanisms these infection and pathogenicity line need many studies and scientific evidences to explained (4–6).

Hemolysis virulence factor ShlA may be considered a major virulence factor in *S. marcescens* pathogenicity using a murine lung infection model and cause hemolytic and cytotoxic effects on erythrocytes, with the aid of an outer membrane protein ShlB (7,8). ShlA is involved in the release of inflammatory mediators, increases the ability of bacteria in high UTI infection, helps in the invasion of the epithelial cell by these bacteria and spreading of a bacteria cell to other host cells (9,10).

Serratia marcescens have virulence factors that make it as capable as an opportunistic pathogen with clinical importance under not clear controlling pathogenesis pathways and poorly understood (11,12). Therefore, this research aimed to study the analysis of a major virulence gene sequencing of *Serratia marcescens* isolated from sputum and burn infection of patients in central Iraqi hospitals in Baghdad including important gene *ShlA* that encodes of toxin cause pore-forming toxin, *PhlA* gene that encode for toxin act as phospholipase with hemolytic and cytolytic activities, *FlhD* gene that encode for toxin lead to flagellar transcriptional regulator.

Materials and Methods

Collection of specimens

The specimens collected include 220 specimens, 100 of which were sputum and 120 burn infection specimens from patients in some hospitals in Baghdad, Iraq, between April and June 2021.

Laboratory diagnosis

All two hundred twenty that were mentioned above arrived at the laboratory and were cultured on blood and MacConkey agar. Then were incubated for 18-24 h at 37 °C. The isolates of *Serratia marcescens* were identified by

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Table 1. Primers of virulence genes in *Serratia marcescens*.

Name of Gene	Type of Primer	Sequences (5′ to 3′)	Annealing Temperature	Reference
PhlA	Forward primer	GGGGACAACAATCTCAGGA	55.4 °C	(14)
	Reverse primer	ACGCCAACAACATACTGCTTG		
ShlA	Forward primer	AGCGTGATCCTCAACGAAGT	55.4 °C	(14)
	Reverse primer	TGCGATTATCCAGAGTGCTG		
FlhD	Forward primer	TGTCGGGATGGGGAATATGG	57 °C	(15)
	Reverse primer	CGATAGCTCTTGCAGTAAATGG		

Table 2. *Serratia marcescens* in sputum an burn infection.

samples	No. of samples	No. of isolates	Percentage
Sputum	100	19	19 %
burn infection	120	21	17.5 %

conventional morphological and biochemical tests (13) and certified by the VITEK- 2 Compact system (Biomerieux/ France) for use in the analysis of virulence gene sequencing.

Extraction bacterial genome

Extraction bacterial genome of all isolates was carried out. The specific primers as shown in Table 1 below amplify all target virulence genes. After the amplification step, gel electrophoresis by using ethidium bromide was done of all amplicon against DNA ladder marker (1500 bp) from Promega USA, then visualized by UV light as shown in Figures 1, 2 and 3 in the results.

Analysis of sequencing virulence genes

Analysis sequencing of virulence genes from *Serratia marcescens* carried out after sending product of PCR of genes (*PhlA, ShlA, FlhD*) to macrogen (Korea) to detect sequences of these genes by machine ABI3730XL, automated sequencer DNA. The results of sequencing these genes were received by Email and certified in NCBI (BLAST). Then explain the analysis of sequencing virulence genes and show variation of *Serratia marcescens* isolates by Geneious version-9. Then document new variations in NCBI.

Results

Laboratory diagnosis

Laboratory diagnosis of these bacteria was done by conventional methods including morphological, and biochemical tests. Then the results of laboratory diagnosis were certified by the VITEK- 2 compact system to show 40 bacterial isolates of *Serratia marcescens* from total samples (220) in percentage (18.18%) in some hospitals in Baghdad as mentioned in Table 2.

Virulence genes in *Serratia marcescens*

Virulence genes (*PhlA, ShlA, FlhD*) in this pathogen were carried out. Firstly, extraction of DNA genes above from all isolates of these bacteria. Then gel electrophoresis process was done by using ethidium bromide stain in a concentration of agarose gel (1.5%). All the isolates of *Serratia marcescens* exhibited found *FlhD* virulence gene and some isolates contained another virulence gene mentioned above against the DNA ladder marker (1500 bp) explained in Figures 1, 2 and 3.

Analysis of virulence genes sequencing

Analysis of virulence genes sequencing from *Serratia marcescens* achieved to all Virulence genes (*PhlA, ShlA, FlhD*) by basic local alignment search tool (BLAST) and Geneious version-9. The results of analysis sequencing exhibited different percentages in genetic identity distance as explained in Tables 3, 4, and 5 which refer to these bacteria new variation in Pathogenicity Island. These results explained the ability of these bacteria to produce different levels of virulence factors that lead to an increase in patho-

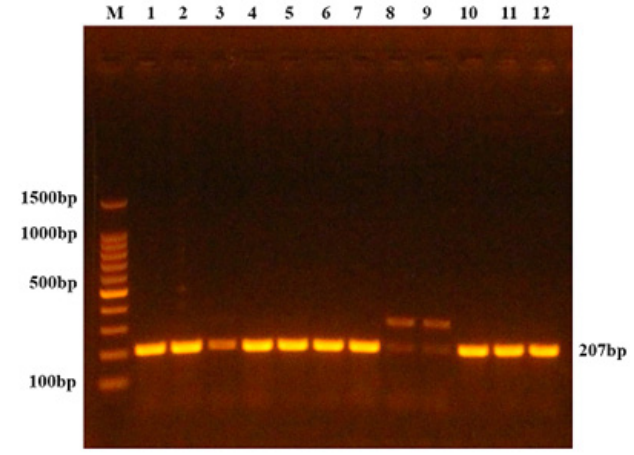

Figure 1. Gel electrophoresis of *Ph1A* virulence gene (207 bp) in *Serratia marcescens*. agarose gel 1.5%, 50 V, 1 hour, DNA ladder (M) 1500 bp.

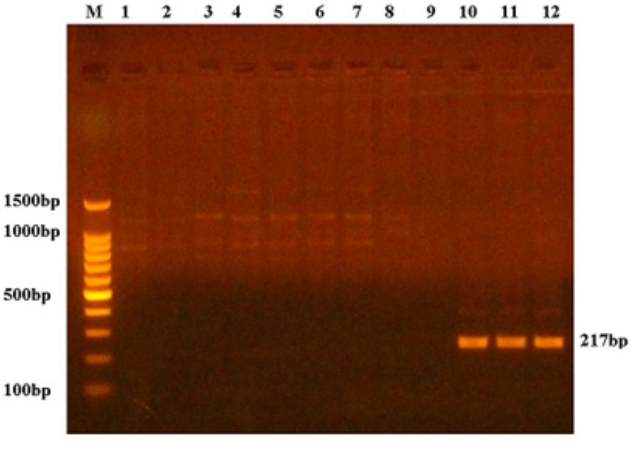

Figure 2. Gel electrophoresis of *Sh1A* virulence gene (217 bp) in *Serratia marcescens*. agarose gel 1.5%, 50 V, 1 hour, DNA ladder (M) 1500 bp.

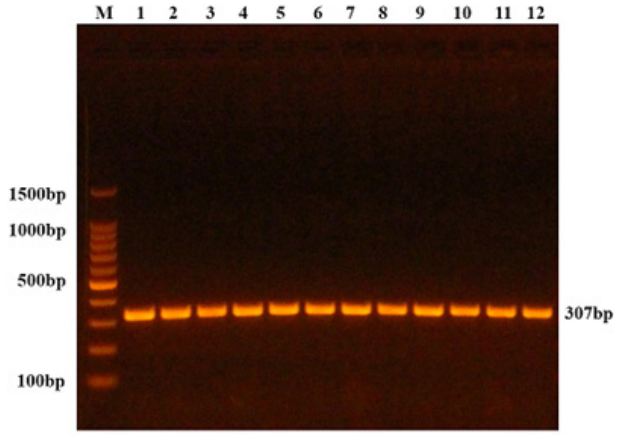

Figure 3. Gel electrophoresis of *FlhD* virulence gene (307 bp) in *Serratia marcescens*. agarose gel 1.5%, 50 V, 1 hour, DNA ladder (M) 1500 bp.

genicity.

Global registration of Iraqi sequences virulence genes

Global registration of Iraqi sequences of virulence genes (*PhlA, ShlA, FlhD*) from *Serratia marcescens* was documented in NCBI to certainly find variation in sequences of these virulence genes in these pathogenic bacteria isolated from hospitals in Baghdad-Iraq. These results refer to found different genetic distance between sequence virulence factors from Iraqi isolates *Serratia marcescens* and those previously documented in NCBI. The accession numbers of virulence sequence genes from Iraqi *Serratia marcescens* were (*Ph1A* virulence gene LC647828.1 & LC647829.1), (*Sh1A* virulence gene LC647830.1 & LC647831.1), (*F1hD* virulence gene LC647826.1, LC647827.1). Results details of virulence sequence genes from Iraqi *Serratia marcescens* are explained in the supplementary Figures 4, 5 and 6.

Discussion

Pathogenicity of *Serratia marcescens* increased through the ability of these pathogenic bacteria to have virulence genes that encode virulence factors like different toxins and hydrolytic enzymes that are capable of these bacteria to invade host defense, host cell destruction, proliferation of bacteria as well as inhibition of host defense border that often lead to death (16–18). *Serratia marcescens* isolated from Iraqi central hospitals like hospitals in the City of Medicine as well as other major hospitals exhibited the ability to produce virulence factors at different levels according to virulence genes in the content of its genome.

All isolates of *S.marcescens* in this study contained the virulence gene *FlhD*, but some isolates of these bacteria contained other virulence genes (*PhlA, ShlA*) in different

Table 3. Percentage of genetic identity distance in sequencing *PhlA* virulence gene.

LC647828		LC647829	MH460878	CP041129	CP060276	CP060483	CP050013
LC647828		97.87	97.14	97.14	97.14	97.14	99.29
LC647829	97.87		99.37	99.37	99.37	99.37	97.16
MH460878	97.14	99.37		100	100	100	97.86
CP041129	97.14	99.37	100		100	100	97.86
CP060276	97.14	99.37	100	100		100	97.86
CP060483	97.14	99.37	100	100		100	97.86
CP050013	99.29	97.16	97.86	97.86	97.86	97.86	

Table 4. Percentage of genetic identity distance in sequencing *ShlA* virulence gene.

LC647830		LC647831	CP053572	CP053927	CP047688	CP047391	CP047691
LC647830		100	98.79	98.18	98.18	98.18	98.18
LC647831	100		98.79	98.18	98.18	98.18	98.18
CP053572	98.79	98.79		96.97	96.97	96.97	96.97
CP053927	98.18	98.18	96.97		98.79	98.79	98.79
CP047688	98.18	98.18	96.97	98.79		100	100
CP047391	98.18	98.18	96.97	98.79	100		100
CP047691	98.18	98.18	96.97	98.79	100	100	

Table 5. Percentage of genetic identity distance in sequencing *FlhD* virulence gene.

LC647826		LC647827	CP059038	CP059036	AP013063	CP026702	CP018923
LC647826		97.08	97.08	97.08	97.81	97.81	97.81
LC647827	97.08		100	100	99.27	99.27	99.27
CP059038	97.08	100		100	99.27	99.27	99.27
CP059036	97.08	100	100		99.27	99.27	99.27
AP013063	97.81	99.27	99.27	99.27		100	100
CP026702	97.81	99.27	99.27	99.27	100		100
CP018923	97.81	99.27	99.27	99.27	100	100	

Footnote: LC647826, LC647827, LC647828, LC647829, LC647830, and LC647831 refer to Iraqi sequences of virulence genes from *Serratia marcescens* that registered in NCBI (Figures 4, 5 and 6).

percentages. These results explained the ability of these pathogens to produce different levels of virulence and variable pathogenicity. Virulence gene *FlhD* encodes for the flagellar transcriptional regulator that plays a role in the biogenesis process of flagella (regulator controlling), formation of biofilm, septation of bacterial cells and gene expression of virulence factors during swarming motility of these bacteria in host cell infection (6,19,20).

The genome of *S.marcescens* has virulence genes *ShlA* that encode for pore-forming toxin and hemolytic activity. This virulence factor is hemolysin/cytolysin considered one type of toxins that cause pore forming in host cells causing binding with specific targets in the host cell membrane leading to unstable permeability of materials through the membrane (21,22). This pore-forming by the Sh1A virulence factor causes an increase in permeability and destruction of the infected host cell. These pathogenic bacteria produce two types of Sh1A virulence factors called Sh1A and Sh1B that are associated with cooperation in the destruction of the membrane of host cells (21,23,24).

Also, these bacteria have *PhlA* virulence gene that encodes for phospholipase with hemolytic and cytolytic activities in nearly the same action manner as the Sh1A virulence factor. Phospholipases different roles lead to increased pathogenicity of these bacteria including two extracellular PLAs, PhlA and PlaA that increase the ability of *Serratia marcescens* in hemolytic and cytolytic activity (25,26). All virulence genes (*PhlA, ShlA, FlhD*) and his product mentioned above are considered major virulence factors and key to the pathogenicity of *Serratia marcescens* (27,28).

Regulation of gene expression of these major virulence factors is very necessary to increase pathogenicity through the quorum sensing mechanism but explaining the mechanism of pathogenesis needs more scientific studies to be understood in complete form (29-31). These intercellular communication systems stimulate the production of virulence factors in *S. marcescens*, also motility as well as the production of biofilm that protects bacteria from mechanisms of defense of the human body (32-34).

Analysis sequencing of these major virulence genes in *S. marcescens* isolated from Iraqi hospitals shown in Tables 3, 4 and 5 explain the total identity of sequencing these virulence genes (*PhlA, ShlA, FlhD*) among local Iraqi bacteria. Also, the results of sequencing analysis showed misidentify or dissimilarity in different levels between Iraqi *S. marcescens* and global strains recorded in NCBI because of environmental and recurrent mutation of bacteria that control of spreading infection and developing high levels of pathogenicity in different countries in the world population. These results are scientific evidence to find a new variation of these virulence genes in Iraqi *S. marcescens*. Therefore, these new variation sequencing registered in the global database in NCBI under accession numbers including (*Ph1A* virulence gene LC647828.1 & LC647829.1), (*Sh1A* virulence gene LC647830.1 & LC647831.1) & (*F1hD* virulence gene LC647826.1, LC647827.1) according to details in the supplementary Figures 4, 5 and 6.

Analysis sequencing of virulence genes (*PhlA, ShlA, FlhD*) in Iraqi isolates *Serratia marcescens* showed top similarity sequences of each virulence gene between local isolates. Also, there is dissimilarity in sequences of virulence genes between Iraqi and global *Serratia marcescens*. This study found new variations in sequences of these virulence genes and registered in NCBI (*Ph1A* virulence gene LC647828.1 & LC647829.1), (*Sh1A* virulence gene LC647830.1 & LC647831.1) & (*F1hD* virulence gene LC647826.1, LC647827.1). The genome of Iraqi *Serratia marcescens* isolates contained these virulence genes in different levels that explain different levels of pathogenicity in these bacteria in comparison with global bacteria.

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None

Interest conflict
The authors declare that they have no conflict of interest.

References

1. Palmer M. The family of thiol-activated, cholesterol-binding cytolysins. Toxicon 2001;39:1681–9.
2. Kurz CL, Chauvet S, Andrès E, Aurouze M, Vallet I, Michel GPF, et al. Virulence factors of the human opportunistic pathogen Serratia marcescens identified by in vivo screening. EMBO J 2003;22:1451–60.
3. Hertle R, Schwarz H. Serratia marcescens internalization and replication in human bladder epithelial cells. BMC Infect Dis 2004;4:1–14.
4. Hertle R. The family of Serratia type pore forming toxins. Curr Protein Pept Sci 2005;6:313–25.
5. Lai H-C, Soo P-C, Wei J-R, Yi W-C, Liaw S-J, Horng Y-T, et al. The RssAB two-component signal transduction system in Serratia marcescens regulates swarming motility and cell envelope architecture in response to exogenous saturated fatty acids. J Bacteriol 2005;187:3407–14.
6. Buffet-Bataillon S, Rabier V, Bétrémieux P, Beuchée A, Bauer M, Pladys P, et al. Outbreak of Serratia marcescens in a neonatal intensive care unit: contaminated unmedicated liquid soap and risk factors. J Hosp Infect 2009;72:17–22.
7. Moradigaravand D, Boinett CJ, Martin V, Peacock SJ, Parkhill J. Recent independent emergence of multiple multidrug-resistant Serratia marcescens clones within the United Kingdom and Ireland. Genome Res 2016;26:1101–9.
8. Vetter L, Schuepfer G, Kuster SP, Rossi M. A hospital-wide outbreak of Serratia marcescens, and Ishikawa's "Fishbone" analysis to support outbreak control. Qual Manag Health Care 2016;25:1.
9. Zingg W, Soulake I, Baud D, Huttner B, Pfister R, Renzi G, et al. Management and investigation of a Serratia marcescens outbreak in a neonatal unit in Switzerland–the role of hand hygiene and whole genome sequencing. Antimicrob Resist Infect Control 2017;6:1–6.
10. Ghaith DM, Mahmoud Zafer M, Ismail DK, Al-Agamy MH, Bohol MFF, Al-Qahtani A, et al. First reported nosocomial outbreak of Serratia marcescens harboring blaIMP-4 and blaVIM-2 in a neonatal intensive care unit in Cairo, Egypt. Infect Drug Resist 2018:2211–7.
11. Phan HTT, Stoesser N, Maciuca IE, Toma F, Szekely E, Flonta M, et al. Illumina short-read and MinION long-read WGS to characterize the molecular epidemiology of an NDM-1 Serratia marcescens outbreak in Romania. J Antimicrob Chemother 2018;73:672–9.
12. Cristina ML, Sartini M, Spagnolo AM. Serratia marcescens infections in neonatal intensive care units (NICUs). Int J Environ Res Public Health 2019;16:610.
13. LM P. Harley JP, Klein DA. Microbiology 2005.
14. Aggarwal C, Paul S, Tripathi V, Paul B, Khan MA. Characteri-

zation of putative virulence factors of Serratia marcescens strain SEN for pathogenesis in Spodoptera litura. J Invertebr Pathol 2017;143:115–23.
15. Salini R, Pandian SK. Interference of quorum sensing in urinary pathogen Serratia marcescens by Anethum graveolens. Pathog Dis 2015;73:ftv038.
16. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6:1–8.
17. Mahlen SD. Serratia infections: from military experiments to current practice. Clin Microbiol Rev 2011;24:755–91.
18. Fedrigo G V, Campoy EM, Di Venanzio G, Colombo MI, García Véscovi E. Serratia marcescens is able to survive and proliferate in autophagic-like vacuoles inside non-phagocytic cells. PLoS One 2011;6:e24054.
19. Castelli ME, Fedrigo G V, Clementín AL, Ielmini MV, Feldman MF, Véscovi EG. Enterobacterial common antigen integrity is a checkpoint for flagellar biogenesis in Serratia marcescens. J Bacteriol 2008;190:213–20.
20. Maragakis LL, Winkler A, Tucker MG, Cosgrove SE, Ross T, Lawson E, et al. Outbreak of multidrug-resistant Serratia marcescens infection in a neonatal intensive care unit. Infect Control Hosp Epidemiol 2008;29:418–23.
21. Lin C-S, Horng J-T, Yang C-H, Tsai Y-H, Su L-H, Wei C-F, et al. RssAB-FlhDC-ShlBA as a major pathogenesis pathway in Serratia marcescens. Infect Immun 2010;78:4870–81.
22. Pramanik A, Könninger U, Selvam A, Braun V. Secretion and activation of the Serratia marcescens hemolysin by structurally defined ShlB mutants. Int J Med Microbiol 2014;304:351–9.
23. McMahon KJ, Castelli ME, Vescovi EG, Feldman MF. Biogenesis of outer membrane vesicles in Serratia marcescens is thermoregulated and can be induced by activation of the Rcs phosphorelay system. J Bacteriol 2012;194:3241–9.
24. Di Venanzio G, Stepanenko TM, Garcia Vescovi E. Serratia marcescens ShlA pore-forming toxin is responsible for early induction of autophagy in host cells and is transcriptionally regulated by RcsB. Infect Immun 2014;82:3542–54.
25. Shimuta K, Ohnishi M, Iyoda S, Gotoh N, Koizumi N, Watanabe H. The hemolytic and cytolytic activities of Serratia marcescens-phospholipase A (PhlA) depend on lysophospholipid production by PhlA. BMC Microbiol 2009;9:1–10.
26. Marques-Pereira C, Proença DN, Morais P V. Genome sequences of Serratia strains revealed common genes in both serratomolides gene clusters. Biology (Basel) 2020;9:482.
27. Ferreira RL, Rezende GS, Damas MSF, Oliveira-Silva M, Pitondo-Silva A, Brito MCA, et al. Characterization of KPC-producing Serratia marcescens in an intensive care unit of a Brazilian tertiary hospital. Front Microbiol 2020;11:956.
28. Khayyat AN, Hegazy WAH, Shaldam MA, Mosbah R, Almalki AJ, Ibrahim TS, et al. Xylitol inhibits growth and blocks virulence in Serratia marcescens. Microorganisms 2021;9:1083.
29. Fekrirad Z, Gattali B, Kashef N. Quorum sensing-regulated functions of Serratia marcescens are reduced by eugenol. Iran J Microbiol 2020;12:451.
30. Jia X, Liu F, Zhao K, Lin J, Fang Y, Cai S, et al. Identification of essential genes associated with prodigiosin production in Serratia marcescens FZSF02. Front Microbiol 2021;12:705853.
31. Li X, Tan X, Zhang J, Zhang J. Complete genome sequences of one prodigiosin-producing Serratia marcescens strain ZPG19. Front Bioeng Biotechnol 2021;9:665077.
32. Ramanathan S, Ravindran D, Arunachalam K, Arumugam VR. Inhibition of quorum sensing-dependent biofilm and virulence genes expression in environmental pathogen Serratia marcescens by petroselinic acid. Antonie Van Leeuwenhoek 2018;111:501–15.
33. Fekrirad Z, Kashef N, Arefian E. Photodynamic inactivation diminishes quorum sensing-mediated virulence factor production and biofilm formation of Serratia marcescens. World J Microbiol Biotechnol 2019;35:1–9.
34. AbdulKarim AT, Hatem AN, Al-Mayah SH. A retrospective study of tick fauna of Iraq – checklist . Scientific Reports in Life Sciences 2023; 4(2): 35–44. https://doi.org/10.5281/zenodo.8277712