Iraqi Journal of Biotechnology, 2025, Vol. 24, No. 3, 166-174

Biofilm Formation by Uropathogenic*Serratiamarcescens*

[1]Nada S. Oudah, [2]Harith J.F. Al-Mathkhury

[1,2] Department of Biology, College of Science, University of Baghdad

Received: July 9, 2024 / Accepted: July 31, 2024 / Published: November 3, 2025

Abstract: Gram-negative nosocomial *Serratiamarcescens* is responsible for severe urinary tract infections (UTIs) in hospitalized individuals. *S. marcescens*'squorum sensing (QS) system regulates biofilm formation. This study aimed to test *S. marcescens*'s capacity to establish biofilms. Out of 150 clinical specimens were collected from patients admitted to Baghdad Medical City, only 23 were positive growth of *S. marcescens*.The *S. marcescens*isolates were idenitifed byCHROMagar*Serratia* and the VITEK-2 system compact. Microplate technique was employed to investigate biofilm formation. DNA extractions were conducted on all positive isolates to confirm their identity through PCR amplification of a fragment of *rplU*gene. *S. marcescens* was identified in all 23 isolates. 11 (47.8%) and 12 (52.2%) isolates were capable of establishing moderate and weak biofilms, respectively. However, none of the isolates was a robust biofilm-former.*Serratiamarcescens* isolates from catheter-associated and UTIs have shown a significant capacity for biofilm formation, indicating potential for UTIs.

Keywords: Biofilm,*rplU*,*Serratiamarcescens*, Uropathogenic.

Corresponding author:(Email:nada.sabbah1602a@sc.uobaghdad.edu.iq).

Introduction

Serratiamarcescens is human pathogen from the *Enterobacteriaceae* family that is gram-negative and opportunistic, *Serratiamarcescens* is responsible for hospital-acquired infections and outbreaks in critically ill or severely immunocompromised patients(1).*S. marcescens*is a pathogen that has the potential to induce a variety of illnesses, such as urinary tract infections (UTI), meningitis, pneumonia, wound infections,septicemia keratitis, and lung infections(2).It is believed that the bloodstream is the most common location for the transmission of infections when the respiratory and digestive systems are compromised. *S. marcescens* is capable of producing virulence factors, such as the capacity to establish biofilms on abiotic or biotic surfaces(3).Furthermore, *S. marcescens* can be isolated from soil samples as well (4).Through a sequence of predetermined stages,*S. marcescens*generate biofilms that essentially consist of cell chains, cell clusters, and filaments, resulting in a highly porous, filamentous biofilm. This biofilm is contingent upon the quorum sensing (QS) system (5).

Labbate *et al.*(6) reported that the process of biofilms developing in *S. marcescens* is genetically regulated. As the biofilm matures, the development of interconnected cell aggregates, bacterial cell assemblages alongside the threadlike cells, and extended threadlike

cells is indicative of the resulting cell and structure differentiation.

A bacterial community can benefit from the formation of biofilms in a variety of ways. Biofilms offer protection against protozoan graze, resistance to a variety of antimicrobials, and defence against host immunity(7).The bacteria's formation of a highly filamentous and porous biofilm, which is composed of filaments andcell chains, is the result of a sequence of distinct stages(8).Protruding organelles, known as fimbriae or pili, are primarily responsible for inert surfaces or living tissue adhesion, entry into host cells, and interactions of bacterium-to-bacterium that result in bacterial aggregation and biofilm formation(9).The pathogenesis of *S. marcescens* is likely influenced by biofilm formation, which is the adhesion of microbes to surfaces such as contact lenses(10).The biofilms growth enables populations of bacteria to survive more effectively in in-hospitable conditions whena hostile immune system or antibiotics are present (11).

The objective of this study was to examine *S. marcescens*'scapacity to establishthe biofilms.

Materials and Methods

Bacterial Isolates

The bacterial isolation was carried out during the period from September to December 2023, a total of 150 different specimens, including mid-stream urine and indwelling urinary catheter, were collected from patients referring to Baghdad Medical City. All the specimens were cultured on the blood agar and MacConkey agar at 37°C for 24 hrs.Colourless colonies (lactose non-fermenters) were chosen for identification of *S. marcescens* as they were cultured on CHROMagar*Serratia*(CHROMagar, France) and incubated at 37ºC for 24 hours. The resulting isolates were then subjected foridentification byVITEK 2 compact system (BioMérieux, France), which utilizes a sophisticated colourimetric approach. *S. marcescens* was identified with a probability of 99% using a Gram-negative (GN) card (12, 13).

Biofilm assay

Biofilm formation was quantified using microplate technique(14). All 23 isolates were cultured in brain heart infusion broth at 37°C for 18 hours. Next, each isolate was diluted in tryptic soy broth (TSB) containing 1% glucose. The 0.5 McFarland standard was used to adjust the cell suspension. Subsequently, addition of 200 µl of the isolated culture to 3 wells of 96-well U-shaped bottom polystyrene microplates. At 37°C, the microplates were incubated for 24 hours and kept covered. The microplates were rinsed twice with distilled water to eliminate loose bacteria, tapped on paper towels to remove any remaining water, and then air-dried. 200 µl of absolute methanol was used to fix each well at room temperature for 20 minutes. Subsequently, application of a volume of 200 µl of 0.1% crystal violet for 15 minutes. The excess dye was eliminated through repeated washing with distilled water. The plates were subsequently air-dried for approximately 30 minutes. 200 µl of 95% ethanol was added to each well after drying, and the crystal violet dye attached to the adherent cells was allowed to dissolve for 10 minutes. This experiment was conducted in triplicate, with the absorbance of bacteria-free weels that contain TSB serving as the negative control.A microplate reader was employed to quantify the samples at an optical density of 600. The classification of bacterial adherence as

shown in (Table 1) was employed to conduct the data simplification and computation. which was derived from the OD_{600} values of individual *S. marcescens* isolates.The cut-off value (ODc) was established as follows (15) : ODc = OD600 of the negative control + 3 standard deviation.

Table (1): Biofilm intensity calculation.

Mean OD_{600}	Biofilm intensity
OD ≤ ODc*	Non-biofilm producer
ODc< OD ≤ 2ODc	Weak
2ODc < OD ≤ 4ODc	Moderate
OD > 4ODc	Strong

Molecular Detection of *Serratiamarcescens*

Extraction of DNA

Presto Mini gDNA bacteria kit (Geneaid, Taiwan) was used for genomic DNA extraction from the bacterial isolates. Theamplification of a fragment of *rpIU*gene was performed using conventional PCR by whichthe extracted DNA,forward primer(5´–GCTTGGAAAAGCTGGACATC-3´) and reverse primer (5´–TACGGTGGTGTTTACGACGA-3´), were used in the PCR master mix with 20 µl as total volume (Table 2). The DNA amplification wascarried out in accordance with the conditions outlined in (Table 3). Subsequently, the amplicons were resolved in a 1.5% agarose gel (16).

Table (2): Components ofmastermix ofconventional PCR.

No.	Component	Concentration	Volume (µl)
1	GoTaq Green Master Mix	2X	10
2	Forward primer (10 µM)	10 µM	1
3	Reverse primer (10 µM)	10 µM	1
4	Nuclease free water	-	6
5	Template DNA	25 ng	2
6	Final volume	-	20

Table (3): PCR amplification program (16).

No.	Step	Temperature (°C)	Time	Cycles
1	Initial denaturation	95	5 min.	1
2	Denaturation	95	45 sec.	40
3	Annealing	57	45 sec.	
4	Extension	72	50 sec.	
5	Final extension	72	5 min.	1
6	Hold temperature	4	∞	1

Statistical analysis

Shapiro-Wilk test was adopted to test the normality. Biofilm data were expressed as mean ± standard deviation, whereas the ANOVA test followed by the least significant difference were adopted for comparing these data. P value ≤ 0.05 was considered significant(17).

Results

Isolation and identification of *Serratiamarcescens*

Out of 150 specimens, 23 (34.5 %) developed positive bacterial growth when cultured on CHROMagar*Serratia*. Plates incubated for 24 hours at 37˚C (Figure 1). The inhibition of the majority of the microorganisms implies a relevant characteristic of CHROMagar*Serratia*(18).The VITEK-2 compact system verified this identification, as all of the isolates were identified as *S. marcescens*. The highest isolation rate was 19 (19%) isolates from 100 mid-stream urine specimens and 4 (8%) isolates from 50 indwelling urinary catheters.

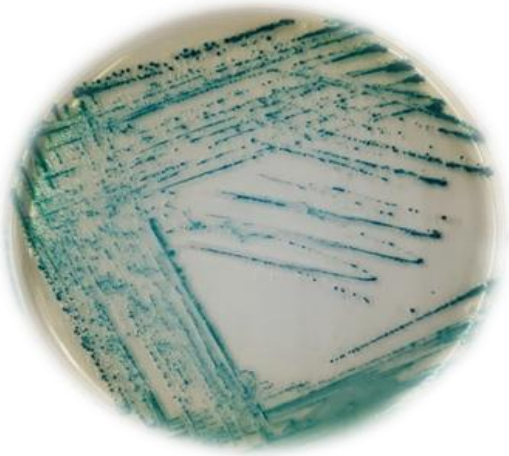

Figure (1):Growth of *Serratiamarcescens* on CHROMagar*Serratia* after incubation at 37˚C for 24 hrs.

Biofilm formation by *Serratiamarcescens*

Biofilm was produced by all twenty-three *S. marcescens* isolates. Nevertheless, a one-way ANOVA revealed substantial differences ($P < 0.05$) among the biofilms that were generated. Furthermore, weak and moderate biofilms were produced by 11 and 12 isolates, respectively. None of the isolates was capable of establishing strong biofilm (Figure 2).

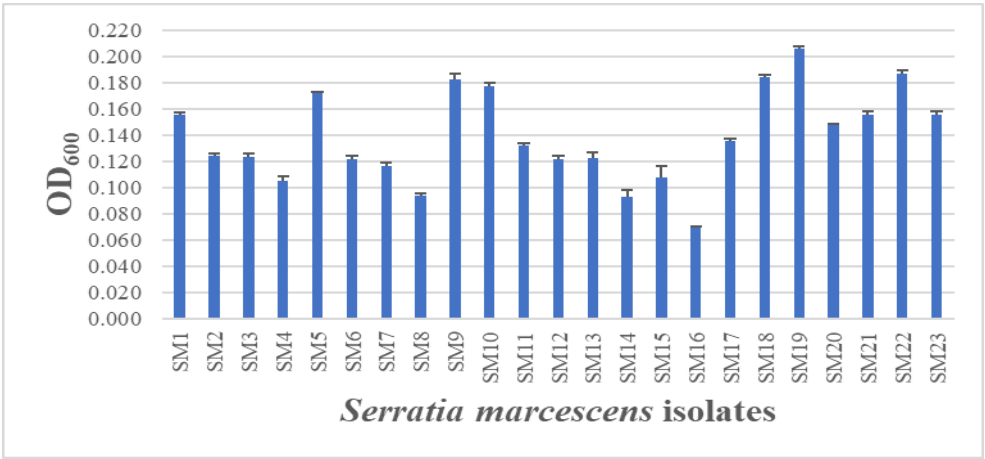

Figure (2): Biofilm formation capacity expressed as OD_{600} of *Serratiamarcescens* isolates. Error bars represent standard deviation. P value = 2.827×10^{-22}, $LSD_{0.05} = 0.016$.

Detection of *rplU*gene

The PCR results (Figure 3) showed that *rpIU*geneexisted in all 23 *S. marcescens*isolates. Markedly, this result may reflect more accurate detection of molecular diagnosis in comparison with CHROMagar*Serratia*, and VITEK 2 compact system methods.

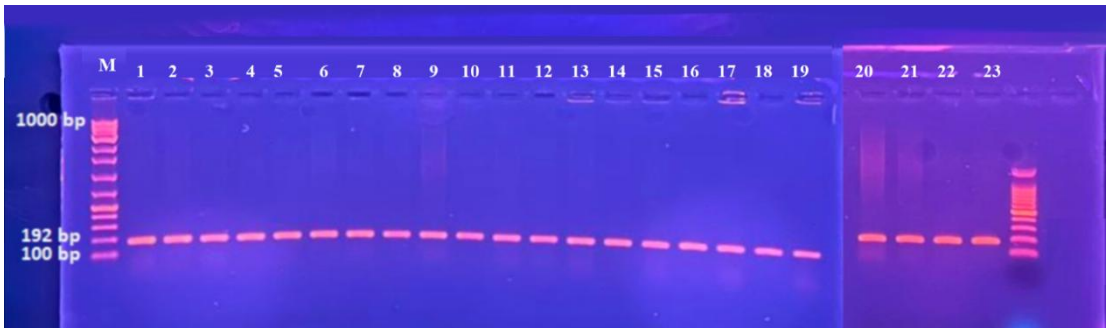

Figure (3): Agarose gel electrophoresis of PCR products using 1.5% at 10 V/cm for 1hr. M:100 bp DNA ladder. Lanes: 1-23 PCR product of *rplU* gene (192bp).

Discussion

A previousrecent study has established the pathogenic nature ofuropathogenic*Serratiamarcescens* in causing urinary tract infections (UTIs)(19),*S. marcescens*, has now beenacknowledged as a frequent cause of nosocomial extra-intestinal infection(20).*S. marcescens*'sinfectious dose can fluctuate based on the individual's susceptibility who is infected. Nevertheless, it's generally believed that a relatively low number of *S. marcescens*can be sufficient to cause an infection in a healthy individual(21). Fever, diarrhea, chills, and abdominal cramps are potential symptoms of *S. marcescens* infection (22). Regardless of the reservoir, the primary method of *S. marcescens* infection transmission is believed to be direct contact between hospital personnel, as evidenced by the relatively widespread distribution of the bacterium among patients with a variety of clinical presentations (23).

Twenty-three isolates of *S. marcescens* (out of 150 clinical specimens) were detected and confirmed using CHROMagar*Serratia*(Figure 1), which agreed with a previous studyby Hatem (12, 13); which identified 27 out of 101 isolates as *S. marcescens* through CHROMagar culturing. The highest percentage of isolation rate was 16 (59.26%) from urine, 8 (29.63%) from blood, 2 (7.41%) from the burn, and one isolate (3.70%) from the wound. The identification of S. *marcescens* in this study was confirmed using the VITEK-2 compact system. In which the identification is in agreement withresearch carried out by Dhaife and Al-Attar (24) documented that from the 120 obtained specimens, four isolates were identified as *S. marcescens* using the VITEK-2 compact system.

Moreover, the present study isolated *S. marcescens* from UTI patients and hospitalized patients with indwelling urinary catheters. Based on the current results, *S. marcescens* isolation from mid-stream urine specimens was higher than those from indwelling catheter-patients. This is in accordance with Potrus(25), Sabaa*et al*.(26), and Al-azzawi*et al*.(27), who all isolated *S. marcescens* from clinical samples in Baghdad. Iraq.Recently,Salim *et al*.(28)documented that 16, 2, and 1 isolates of *S. marcescens* were isolated from UTI, wound infections, and respiratory tract infections, respectively.

The present study confirms the importance of biofilm as an important virulence factor that characterizes *S. marcescens* clinical isolates causing UTI. Through a signal-mediated QS mechanism, *S. marcescens* produces biofilm and releases a variety of virulence factors(29). This research assessed biofilm formation and it shows *S. marcescens*'s ability to detect biofilm formation using a microtiter plate.The precise mechanism by which *S. marcescens* attaches to both (biotic and abiotic surfaces) remains unknown, despite the extensive understanding of its ability to adhere to various substrates (30).

The current results found that 11 and 12 isolates produced weak and moderate biofilms, respectively. While none wasbiofilm non-producer. These data are similar to a recent study conducted by Al-Fayyadh*et al*(15), as they indicated that fifteen and thirty-five isolates, respectivelywere weak and moderate biofilm-former. None of which were strong biofilm-forming. Recently, Salim (28) demonstrated that 18 *S. marcescens* isolates had 100% biofilm production, with 16 (88.88%) isolates producing weak biofilm and 2 (11.11%) isolates forming moderate biofilms.

All the twenty-three *S. marcescens* isolated in this research detect the presence of the housekeeping gene (*rplU*). Similarly, Al-Fayyadh(15)conducted research that utilized the *rplU* gene as a housekeeping gene. A fragment of*rplU* gene was detected in all 50 (16.3%) *S. marcescens* isolates from 350 specimens. Commonly, housekeeping genes (HKG) are characterized as "essential for cellular existence regardless of their specific function in the tissue or organism" and "stably expressed irrespective of tissue type, developmental stage, cell cycle state, or external signal." (31). HKG are genes that are ubiquitously expressed in all tissue compartments and cell types, irrespective of their developmental stage, physiological condition, and exposure to external stimuli (32). HKG genes are frequently employed as reference genes in gene expression studies to normalize the expression levels of target genes, as they are essential for the maintenance of basic cellular functions. The internal control was established as the *rplU* gene of *S. marcescens* (33, 34). The internal reference gene was *rplU*, and the calibrator was wild-type cells(35).

Exposuretomedical interventions, protracted hospitalization, compromised clinical conditions of patients, and increased frequency and intensity of direct contact with healthcare personnel are all factors that influence the opportunistic nature of this bacterium. The importance of meticulous surveillance and preventative measures to mitigate the transmission of this pathogen is underscored by the heightened vulnerability of patients to *S. marcescens* infections in healthcare environments (36). *S. marcescens* produces biofilms that are distinct from those generated by other conventional bacteria that form biofilm, including *E. coli* and *P. aeruginosa*, in which undifferentiated cells are arranged in microcolonies (37). This pathogen is implicated in (2.5–7.7) % of catheter-associated infections, including central line-associated bloodstream infections and catheter-associated UTI. The formation of biofilms is facilitated by the survival advantage that bacterial colonization of catheters provides (38).

Ultimately, it can be stated that QS is an established mechanism in pathogenic and opportunistic bacteria that modulates biofilm, motility, secreted

toxins, enzymes, and different virulence determinants. Because there is little constraint on bacterial growth, targeting bacterial pathogenicity was much less likely to produce resistance.Alternatively, virulence suppression weakens pathogens and improves the immune system's capacity to destroy them (2).

Conclusion

The results indicated that *S. marcescens* isolates collected from patients with catheter-associated infections and urinary tract infections demonstrate a substantial capacity for biofilm formation. The extensive dissemination of a highly genotoxic strain in urinary tract infections in Iraq is a significant indication of the occurrence of biofilm from isolates. This specific strain has the potential to lead to the development of urinary tract infections. Nevertheless, additional research is necessary to restrict the capacity of *S. marcescens* to form biofilms.

References

1. Erat, T.; H. Ozdemir; A. Yahsi; T. TURAL KARA; E. Azapagasi; T. KENDİRLİ; E. Ince; B. Atasay; E. Ünal, and D. Aysev. (2022). Bloodstream Infections Caused by *Serratiamarcescens*: Have a High Prevalence of ESBL and Carbapenemase Production in Pediatric Patients. Journal of pediatric infection (3).
2. Abbas, H.A. and W.A.H. Hegazy. (2020). Repurposing anti-diabetic drug “Sitagliptin” as a novel virulence attenuating agent in *Serratiamarcescens*. PLOS ONE, 15(4), e0231625.
3. Hamza, A.A. and M.K.A. Al-Hassani. (2023). Biofilm Formation, Antimicrobial Resistance in *Serratiamarcescens* Isolated from Different Clinical Cases. Journal of Population Therapeutics and Clinical Pharmacology, 30(7), 460-465.
4. Abboodi, A.; K. Al-Jobori; M. Jumaa, and A. Sultan. (2021). Isolation of Multi-Trait Plant Growth-Promoting *Serratiamarcescens* and Evaluation of Growth- Promoting Effects on Wheat Plant under Salinity Stress. 20, 78-95.
5. Zhou, J.W.; P.C. Ji; C.Y. Wang; Y.J. Yang; X.Y. Zhao; H.Z. Tang, and S.R. Tang. (2023). Synergistic effect of propyl gallate and antibiotics against biofilms of *Serratiamarcescens* and Erwiniacarotovora in vitro. LWT, 173, 114258.
6. Labbate, M.; Y. Queck Shu; S. Koh Kai; A. Rice Scott; M. Givskov, and S. Kjelleberg. (2004). Quorum Sensing-Controlled Biofilm Development in *Serratialiquefaciens* MG1. Journal of Bacteriology, 186(3), 692-698.
7. Anderson, G.G. and G.A. O'Toole. (2008). Innate and induced resistance mechanisms of bacterial biofilms. Bacterial biofilms, 85-105.
8. Liu, D.-R.; Q.-X. Yan; Z.-B. Zou; C.-L. Xie; X.-W. Yang, and A.-Q. Jia. (2023). Cladosporiumsphaerospermum extract inhibits quorum sensing associated virulence factors of *Serratiamarcescens*. Biofilm, 6, 100146.
9. Proft, T. and E.N. Baker. (2009). Pili in Gram-negative and Gram-positive bacteria—structure, assembly and their role in disease. Cellular and molecular life sciences, 66, 613-635.
10. Hume, E.B.H. and M.D.P. Willcox, Emergence of *Serratiamarcescens* as an ocular surface pathogen. 2004. p. 475-477.
11. Zhao, A., Sun, J., & Liu, Y. (2023). Understanding bacterial biofilms: From definition to treatment strategies. Frontiers in cellular and infection microbiology, 13, 1137947.
12. Rasheed, M.N.; S.H. Mohaisen, and K. Khtaua. (2020). Isolation, Molecular Identification and Influence of Incubation Period on Hemolysine Gene Expression in *Serratiamarcescens* Local Isolates. Int J Nat Eng Sci, 14(1), 3-7.
13. Mawahb Hatem Mons , R.A.A. (2022). Effect of purified hemolysin from S. marcescenson some pathogenic isolates and itis inhibitoryeffect on pathogenic bacteria. International Journal of Special Education, 37.
14. Khayyat, A.N.; W.A.H. Hegazy; M.A. Shaldam; R. Mosbah; A.J. Almalki; T.S. Ibrahim; M.T. Khayat; E.S. Khafagy; W.E. Soliman, and H.A. Abbas. (2021). Xylitol inhibits growth and blocks virulence in *Serratiamarcescens*. Microorganisms, 9(5), 1083.
15. Al-Fayyadh, Z.H.; A.M. Turki, and H.J.F. Al-Mathkhury. (2023). Glyceryl Trinitrate, a Vasodilating Drug Acts as an Antibiofilm

Agent in *Serratiamarcescens*. J Contemp Med Sci| Vol, 9(3), 175-178.
16. Srinivasan, R.; R. Mohankumar; A. Kannappan; V. Karthick Raja; G. Archunan; S. Karutha Pandian; K. Ruckmani, and A. Veera Ravi. (2017). Exploring the Anti-quorum Sensing and Antibiofilm Efficacy of Phytol against *Serratiamarcescens* Associated Acute Pyelonephritis Infection in Wistar Rats. Frontiers in cellular and infection microbiology, 7.
17. Pagano, M., Gauvreau, K., & Mattie, H. (2022). Principles of biostatistics. Chapman and Hall/CRC.
18. Perez-Viso, B.; S. Aracil-Gisbert; T.M. Coque; R. Del Campo; P. Ruiz-Garbajosa, and R. Cantón. (2021). Evaluation of CHROMagar™-*Serratia* agar, a new chromogenic medium for the detection and isolation of *Serratiamarcescens*. European Journal of Clinical Microbiology & Infectious Diseases, 40(12), 2593-2596.
19. Gery, A.; A. Mouet; F. Gravey; M. Fines-Guyon; F. Guerin; F. Ethuin; F. Borgey; J. Lubrano, and S. Le Hello. (2021). Investigation of *Serratiamarcescens* surgical site infection outbreak associated with peroperative ultrasonography probe. Journal of Hospital Infection, 111, 184-188.
20. Kannan, S.; J. Balakrishnan; A. Govindasamy, and R. Arunagiri. (2022). New insights into the antibacterial mode of action of quercetin against uropathogen*Serratiamarcescens* in-vivo and in-vitro. Scientific Reports, 12(1), 21912.
21. Attman, E.; P. Korhonen; O. Tammela; R. Vuento; J. Aittoniemi; J. Syrjanen; E. Mattila; M. Osterblad, and R. Huttunen. (2018). A *Serratiamarcescens* outbreak in a neonatal intensive care unit was successfully managed by rapid hospital hygiene interventions and screening. ActaPaediatrica, 107(3), 425-429.
22. Tomanic, D.; B. Bozin; N. Kladar; J. Stanojević; I. Cabarkapa; N. Stilinovic; J. Apić; D.D. Bozic, and Z. Kovacevic. (2022). Environmental bovine mastitis pathogens: prevalence, antimicrobial susceptibility, and sensitivity to Thymus vulgaris L., Thymus serpyllum L., and Origanum vulgare L. essential oils. Antibiotics, 11(8), 1077.
23. Mahlen, S.D. (2011). *Serratia* infections: from military experiments to current practice. ClinMicrobiol Rev, 24(4), 755-91.
24. Dhaife, S.S. and N.S. Al-Attar. (2021). Evaluation the Effect of Prodigiosin on Algd Expression in Clinical Isolates of Pseudomonas aeruginosa. Plant Archives, 21(1), 826-834.
25. Potrus, W.M. (2009). Ascorbic acid induced loss of an antibiotic resistance plasmid in *Serratiamarcescens*. Iraqi Journal of Science, 50(1), 37-42.
26. Sabaa, H.S.; N.R. Mohammed, and R.S. Abd. (2017). Effect of Gamma, Beta, Alpha irradiation and effect of Semiconductor laser on *Serratiamarcescens* (in vitro). Iraqi Journal of Biotechnology (Iraqi.j. Biotech.), 16(4).
27. Al-azzawi, R.M.; A. Raheem, and R. Ghizar. (2012). Extraction and partial purification of Protease from local *Serratiamarcescens* isolate. Iraqi Journal of biotechnology, 10(2), 261-272.
28. Salim, W.M.; L.H. Al-bayati, and H.K. Dabis. (2024). Phenotypic and Genotypic Detection of *Serratiamarcescens* Efflux Pumps, Biofilms Formation, and Antimicrobial Resistance Properties in Different Clinical Cases in Wasit Province, Iraq. Romanian Journal of Diabetes, 31(1), 473-491.
29. Fekrirad, Z., Gattali, B., & Kashef, N. (2020). Quorum sensing-regulated functions of *Serratiamarcescens* are reduced by eugenol. Iranian journal of microbiology, 12(5), 451–459.
30. Koo, H. and K.M. Yamada. (2016). Dynamic cell–matrix interactions modulate microbial biofilm and tissue 3D microenvironments. Current opinion in cell biology, 42, 102-112.
31. Joshi, C.J.; W. Ke; A. Drangowska-Way; E.J. O'Rourke, and N.E. Lewis. (2022). What are housekeeping genes? PLoSComputBiol, 18(7), e1010295.
32. Chang, C.W.; W.C. Cheng; C.R. Chen; W.Y. Shu; M.L. Tsai; C.L. Huang, and I.C. Hsu. (2011). Identification of human housekeeping genes and tissue-selective genes by microarray meta-analysis. PloS one, 6(7), e22859.
33. da Conceição Braga, L.; B.Ô.P. Gonçalves; P.L. Coelho; A.L. da Silva Filho, and L.M. Silva. (2022). Identification of best housekeeping genes for the normalization of RT-qPCR in human cell lines. ActaHistochemica, 124(1), 151821.
34. Wang, J.; J. Yang; P. Durairaj; W. Wang; D. Wei; S. Tang; H. Liu; D. Wang, and A.-Q. Jia. (2024). Discovery of β-nitrostyrene derivatives as potential quorum sensing

inhibitors for biofilm inhibition and antivirulence factor therapeutics against *Serratiamarcescens*. mLife, 3(3), 445-458.
35. Shanks Robert, M.Q.; A. Stella Nicholas; J. Kalivoda Eric; R. Doe Megan; M. O'Dee Dawn; L. Lathrop Kira; L. Guo Feng, and J. Nau Gerard. (2007). A *Serratiamarcescens*OxyR Homolog Mediates Surface Attachment and Biofilm Formation. Journal of Bacteriology, 189(20), 7262-7272.
36. Cosimato, I.; B. Santella; S. Rufolo; P. Sabatini; M. Galdiero; M. Capunzo; G. Boccia; V. Folliero, and G.J.A. Franci. (2024). Current Epidemiological Status and Antibiotic Resistance Profile of *Serratiamarcescens*. 13(4), 323.
37. Reisner, A.; J.A. Haagensen; M.A. Schembri; E.L. Zechner, and S. Molin. (2003). Development and maturation of *Escherichia coli* K-12 biofilms. Molecular microbiology, 48(4), 933-946.
38. Bukhari, S.Z.; A. Banjar; S. Baghdadi; B. Baltow; A. Ashshi, and W. Hussain. (2014). Central line associated blood stream infection rate after intervention and comparing outcome with national healthcare safety network and international nosocomial infection control consortium data. Annals of Medical and Health Sciences Research, 4(5), 682-686.