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Molecular detection of virulence genes of *Serratia marcescens* isolates from diverse clinical sources

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Abstract

Serratia marcescens is a rare opportunistic pathogen that produces a red pigment and possess a serious threat to vulnerable people in healthcare settings. This study aimed to identify the virulence genes (*cnfI, sat, hlyA, PhIA, ShIA, ShIB, swr, and sepA*) in clinical isolates of *S. marcescens*. For that purpose, 152 samples were collected from various clinical sources including burns, wounds, and urinary tract infections. Cultural characteristics and biochemical tests were relied upon for the initial diagnosis. The diagnosis was confirmed using the Vitek-2 system and the final determination for *S. marcescens* was achieved via sequencing of the 16S rRNA. Only 5/152 (3.28%) samples were confirmed as *S. marcescens* via 16S rRNA sequencing. Out of 8 virulence factors studied, only *hlya* and *phIA* were detected 60% and 80% were detected, respectively. The number of infections with *S. marcescens* is constantly increasing in Iraq and determining the virulence genes is crucial in order to identify the spread of virulent strains in patients.

***Keywords*:** *Serratia marcescens, PhIA*, 16S rRNA, PCR.
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1. Introduction

Serratia marcescens is a facultative anaerobic gram-negative bacilli. It is motile and belongs to the intestinal microbiota family. It is spread in nature, and can be isolated from various sources including humans, animals, water, soil, insects, and vertebrate gastrointestinal tract [1]. *S. marcescens* is a saprophytic bacterium capable of withstanding many different environmental conditions with a thermal range from (10-40 °C) and pH between (5-9) [2]. *S. marcescens* also produces extracellular enzymes such as DNase, gelatinase and lipase [3]. It has the ability to produce catalase but does not have the effectiveness for producing oxidase [4-6].

Some strains of *S. marcescens* produce the pigment prodigiosin, which causes the red or pink appearance of colonies on nutrient agar [7, 8].

The pathogenicity of *S. marcescens* depends on the extracellular secreted enzymes, including proteases, fimbria, lipopolysaccharide (LPS) and ShlA hemolysin. These enzymes are considered virulence factors [2, 9]. For example, hemolysins are produced by various pathogenic bacteria and have been proposed to be responsible for their pathogenesis [10, 11]. One type of hemolysin, cytolysin is a group of pore-forming toxins. Cytolysin is typically formed as a homo-oligomer that is integrated into the cell membrane, thereby changing the cell permeability and leading to cell death. Hemolysin is another type of virulence factors,

these enzymes have phospholipase C activity [12]. Phospholipise PhlA the other virulence factors is responsible for the lecithinase activity of *S. marcescens* [13]. PhlA is cytotoxic when added to epithelial cells and exhibits hemolytic activity, which is due to the accumulation of lysophospholipid cleavage products that can cause membrane instability of target cells [14,15]. The current study aimed to determine the spread of Enterobacteriaceae virulence genes from *S. marcescens* strains isolated from pathogenic specimens using conventional PCR.

2. Materials and methods

Sample collection

152 samples were collected from patients suffering from burn infections, wound infections and urinary tract infections during the period from February to June 2023. The samples were collected from Al-Salam Teaching Hospital, Mosul General Hospital, Al-Jumhuriya Educational Hospital, and Mosul Specialized Center for Burns and Cosmetic Treatment in Mosul city.

Isolation and identification

S. marcescens isolates were identified depending on its morphological characteristics on MacConkey, nutrient and DNase agar, in addition to other specific biochemical tests [16]. Identification was further confirmed by VITEK-2 system (bioMérieux, France) as well as 16S rRNA gene sequencing [17]. The primers 27F: AGAGTTTGATCMTGGCTCAG and 1522R: AAGGAGGTGATCCARCCGA were used for the amplification of the 1495bp DNA fragment of the 16S rRNA region [18]. Amplification of 16S rRNA was performed in a DNA thermal cycler, with the following cycling program; Initial denaturation for 3 minutes at 95 ºC; followed by 30 cycles including denaturation for 45 seconds at 95 ºC, annealing for 30 seconds at 55 ºC, extension for 1.5 minute at 72 ºC; and a final extension step for 3 minutes at 72ºC [19].

DNA extraction:

Genomic DNA was extracted using the DNA Extraction Kit provided by (Geneaid), steps for extractions was done according to the manufacturer's instructions.

Molecular detection of some virulence genes

Gene	Protein name	Size (bp)	Primer Sequence 5'-3'
cnf1	cytotoxic necrotizing factor type 1	498	F′AATGGAGTTTCCTATGCAGGAG3′ R′CATTCAGAGTCCTGCCCTCATTATT3′
sat	secreted autotransporter toxin	779	F′GGTATTGATATCTCCGGTGAAC3′ R′ATAGCCGCCTGACATCAGTAAT3′
hly	hemolysin	350	F′AACAASGATAAGCACTGTTCTGGCT3′ R′ACCATATAAGCGGTCATTCCCTTCA3
swr	Serrawittin	1100	F′CCGTGTGGCTAGACCAATCT3′ R′GAACGAAGGTGTGTTTCTGCC3′
phlA	phospholipase A	1450	F′GGGGACAACAATCTCAGGA3′ R′ACGCCAACAACATACTGCTTG3′
shlA	Serratia marcescens haemolysin A	480	F′AGCGTGATCCTCAACGAAGT3′ R′TGCGATTATCCAGAGTGCTG3′
shlB	Serratia marcescens haemolysin B	3100	F′AACAGTGCATCAGCAGCAAC3′ R′GCAGCGAATAGAGCAGGGTA3′
sepA	serratiopeptidase	729	F′GGATAATCAGGTGTCAGCCAA3′ R′GGGCATCATTGAGTCTTGGAT3′

Table 1. Primers used in this study showing the estimated size of each ampilcon.

Primers for eight different virulence genes (table 1) specific for Enterobacteriaceae and *S. marcescens* were used to search for corresponding genes in *S. marcescens* using conventional PCR. These genes were: *shlA, shlB, phlA, swr, sepA, cnf1, sat* and *hly*. Table 1 shows the size of the virulence genes and the sequence of the primers used in PCR reactions.

3. Results and discussion

Only 5 out of the 152 total samples isolated were identified as *S. marcescens* according to all identification steps used. The distribution of *S. marcescens* according to sample types were as follows: UTI 3/68 (4.41%), wound infection 1/46 (2.17%), burn infection 1/38 (2.63). According to our findings, the isolation and diagnostic rate of *S. marcescens* among other bacterial species was 3.28%. The findings further corroborate the prevalence and existence of this bacterium in all sources of isolation, with a variation in the proportion of isolates from each source.

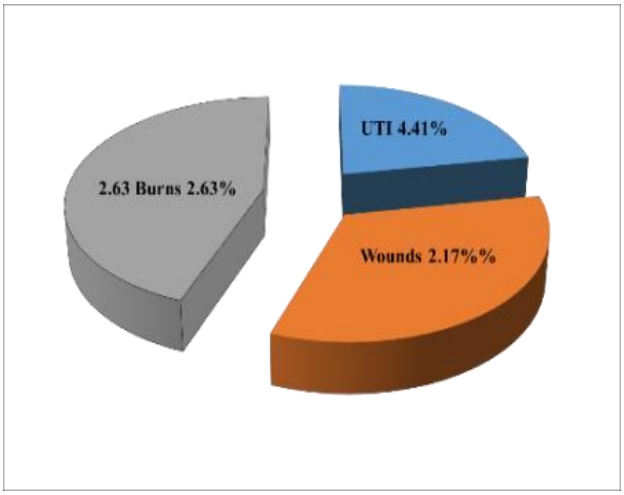

Figure 1. Distribution of *S. marcescens* among the rest of the bacterial species in the isolated infections

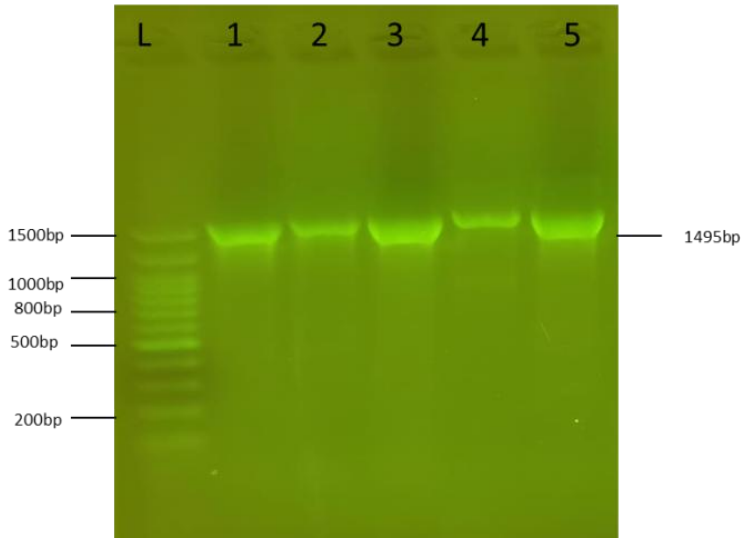

Figure 2. PCR amplification of the 1495bp 16S rRNA fragment from *S. marcescens* isolates. L:100bp DNA ladder, 1-5: *S. marcescens* isolate 1-5.

The biochemical tests and VITEK 2 system was used to confirm identification of *S. marcescens*. On MacConkey agar *S. marcescens* colonies were small, pale and non-lactose

fermenters [7]. Colonies were circular convex with a smooth surface on nutrient agar [6]. The biochemical characteristics of *S. marcescens* were negative for oxidase and positive for catalase. DNase agar (without indicator) showed clear zones around colonies within 5 minutes after HCL addition.

Identification of isolates was confirmed by sequencing their 16S rRNA genes. The PCR results showed that the primers amplified the universal 1495bp band that existed in all five *S. marcescens* isolates (figure 2). The amplicons were extracted from the agarose gel using the PCR clean-up system (Promega/USA), then sent for DNA sequencing at Psomagen sequencing company (USA). The sequences obtained were BLAST alignment in NCBI and showed high identity to *S. marcescens* strains. The isolates were submitted in Gene Bank and given the accession numbers OR272225- OR272227.

Analyzing five *S. marcescens* isolates for the presence of virulence genes revealed the presence of *phIA* in 80% and *hly* in 60% of the isolates. The remaining six virulence genes (*shlA, shlB, swr, sepA, cnf1,* and *sat*) were not detected. Figure 3 shows the gel electrophoresis picture of *hly* in three isolates with a size of 123 bp.

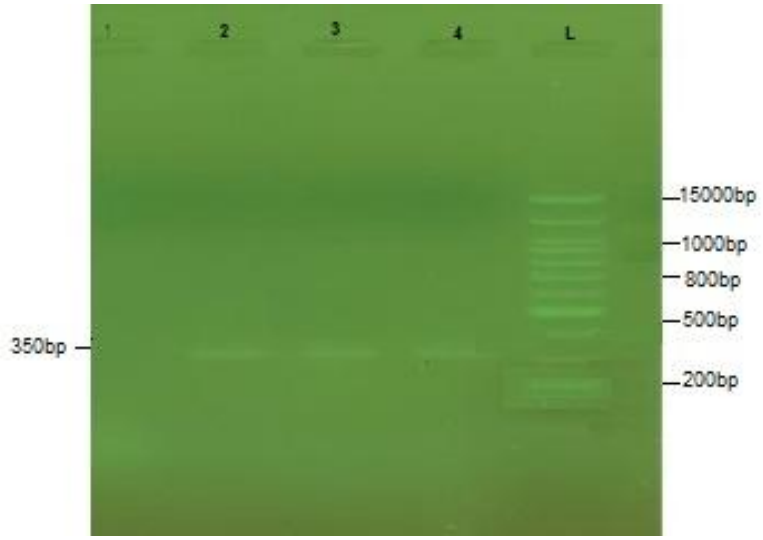

Figure 3. PCR amplification of the *hly* gene from *S. marcescens* isolates. L:100bp DNA ladder, 1-4: *S. marcescens* isolate 1-4.

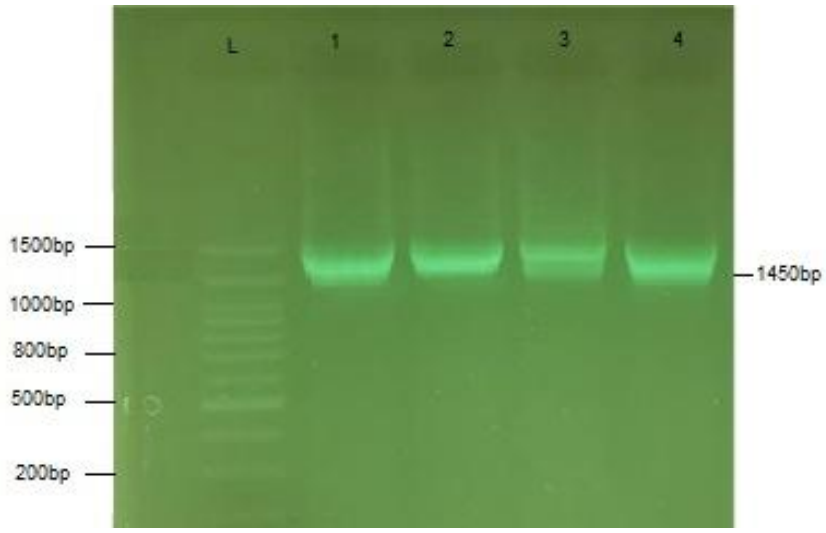

Figure 4. PCR amplification of the 1450bp *phIA* gene from *S. marcescens* isolates. L:100bp DNA ladder, 1-4: *S. marcescens* isolate 1-4.

The gel electrophoreses of *PhIA* for four isolates of *S. marcescens* are shown in Figure (4). The *PhIA* size is 1000 bps.

S. marcescens is a common opportunistic pathogen in hospitalized patients. *Serratia* spp. is usually non-pigmented and causes pneumonia, bacteremia, and endocarditis specifically in narcotics addicts. The isolation of *S. marscecens* is most common in UTI, followed by burns and wounds. Our findings are supported by earlier research [19]. Although *S. marcescens* is recognized to cause UTI, mainly in health care settings and in patients with urinary catheters, the bacterial variables implicated in UTI remain unknown for this organism [20].

The diagnostic findings for the five bacterial isolates were received and firmly verified that they were *S. marcescens*, particularly because they had 98% similarity to the *S. marcescens* registered internationally inside NCBI. The 16S rRNA gene was used to confirm identification of *S. marcescens* isolates. 16S rRNA has a truncation length of 1495 bps. Observations reveal that the nucleotide sequence is not mutable and only slowly varied over time [21].

PhIA was found in four of the isolates under investigation, and this finding was consistent with earlier researches [22, 23]. Given the numerous well-characterized instances of secreted phospholipases with involvement in bacterial virulence, the possibility of *PhlA* contributing to *S. marcescens* pathogenicity is an appealing concept [24]. However, the *hly* has been found in three different bacterial clones.

The rest of the genes did not appear during molecular diagnosis. The loss of virulence factor genes in bacteria is a multifaceted phenomenon documented in the scientific literature. Spontaneous mutations, genetic recombination, and selective pressures play pivotal roles in this process [25, 26]. Environmental influences, host immunological reactions, and competition with other microbes all impose selective pressures on bacterial genomes, resulting to adaptive alterations [27]. The loss of plasmids and transposon excision are examples of horizontal gene transfer processes that further deplete virulence components in bacterial populations [23, 28]. Furthermore, the loss of virulence factors has been related to chromosomal rearrangements, including deletions and rearrangements [29]. Bacterial virulence factors evolve as a result of dynamic interactions fostered by long-term host-pathogen coevolution, thereby changes in their sequence might prevent the primers from binding to their specific sites [30]. Future studies are required to sequence genomes from local *Serratia* isolates and study the variations in virulence genes if any and identify if such changes prevent primer annealing.

4. Conclusion

In Iraq, the number of infections with *S. marcescens* bacterium is steadily growing. Identifying virulence factor genes is critical for detecting mutations. Two virulence factor genes out of eight were found among the isolated strains of *S. marcescens*. The discovery of novel strains of these bacteria alerts, cautions, and draws the attention of the health system.

Authors' Declaration

The authors declare no conflict of interests regarding the publication of this article.

References

1. Z. Kahrarian, S. Khazayel, A. Tajehmiri, Asian Journal of Research in Medicine and Medical Science **1**(1) (2019) 18.
2. A. Hejazi, and F.R. Falkiner, Journal of medical microbiology **46**(11) (1997) 903.
3. R. Zivkovic Zaric, M. Zaric, M. Sekulic, N. Zornic, J. Nesic, V. Rosic, T. Vulovic, M. Spasic, M. Vuleta, J. Jovanovic, D. Jovanovic, S. Jakovljevic, P. Canovic, Antibiotics **12**(2) (2023) 367.

4. M.H. Abd-Alla, S.R. Bashandy, S. Schnell, S. Ratering, Phytoparasitica **39**(2) (2011) 175.
5. E.W. Koneman, W.C. Winn, S.D. Allen, W.M. Janda, G.W. Procop, P.C. Schreckenberger, G.L. Woods, Color Atlas & Textbook of Diagnostic Microbiology.6th Edition, Lippincot Williams &Wilkins, Philadelphia, USA (2006).
6. F. Grimont, and P.A.D. Grimont, The prokaryotes **6** (2006) 197.
7. R.L. Ferreira, G.S. Rezende, M.S.F. Damas, M. Oliveira-Silva, A. Pitondo-Silva, M.C.A. Brito, E. Leonardecz, F.R. de Góes, E.B. Campanini, I. Malavazi, A.F. da Cunha, M.D.S. Pranchevicius, Frontiers in microbiology **11** (2020) 956.
8. D. Kljakić, M.Z. Milosavljević, M. Jovanović, V.Č. Popović, S. Raičević, Open medicine (Wars) **16**(1) (2020) 81.
9. R. Hertle, Current protein & peptide science **6**(4) (2005) 313.
10. M. Palmer, Toxicon: official journal of the International Society on Toxinology **39**(11) (2001) 1681.
11. D.H. Walker, H.M. Feng, V.L. Popov, The American journal of tropical medicine and hygiene **65**(6) (2001) 936.
12. J. Sakurai, M. Nagahama, M. Oda, Journal of biochemistry **136**(5) (2004) 569.
13. M. Givskov, L. Olsen, S. Molin, Journal of bacteriology **170**(12) (1988) 5855.
14. J.K. Song, M.K. Kim, J.S. Rhee, Journal of biotechnology **72**(1-2) (1999) 103.
15. M. Labbate, H. Zhu, L. Thung, R. Bandara, M.R. Larsen, M.D. Willcox, M. Givskov, S. A. Rice, S. Kjelleberg, Journal of bacteriology **189**(7) (2007) 2702.
16. G.M. Garrity, J. Bell, G. Lilburn, Taxonomic Outline of the Prokaryotes. In: Bergey's manual of Systemic Bacteriology, 2nd ed., Berlin Heidelberg, New York (2005).
17. A.M. Khaleel, R.M. Faisal, H.A. Altaii, Revista Bionatura **8**(3) (2023) 113.
18. A.M. Khaleel, R.M. Faisal, H.A. Altaii, Malaysian Journal of Microbiology **19**(2) (2023) 115.
19. R. Abdulrazzaq, R. Faisal, Journal of Life and Bio Sciences Research **3**(01) (2022) 01.
20. L.H. Su, J.T. Ou, H.S. Leu, P.C. Chiang, Y.P. Chiu, J.H. Chia, A.J. Kuo, C.H. Chiu, C. Chu, T.L. Wu, C.F. Sun, T.V. Riley, B.J. Chang, Infection Control Group, Journal of clinical microbiology **41**(10) (2003) 4726.
21. J.E. Clarridge 3rd, Clinical microbiology reviews **17**(4) (2004) 840.
22. K. Shimuta, M. Ohnishi, S. Iyoda, N. Gotoh, N. Koizumi, H. Watanabe, BMC microbiology **9** (2009) 1.
24. J. Hacker, E. Carniel, EMBO reports **21**(51) (2001) 376.
25. M.T. Anderson, L.A. Mitchell, H.L.T. Mobley, Journal of bacteriology **199**(16) (2017) e00159-17.
26. M. Flores-Díaz, L. Monturiol-Gross, C. Naylor, A. Alape-Girón, A. Flieger, Microbiology and molecular biology reviews: MMBR **80**(3) (2016) 597.
27. E. Dadachova, A. Casadevall, Current opinion in microbiology **11**(6) (2008) 525.
28. J. Wiedenbeck, F.M. Cohan, FEMS microbiology reviews **35**(5) (2011) 957.
29. M. Diard, E. Bakkeren, J.K. Cornuault, K. Moor, A. Hausmann, M.E. Sellin, C. Loverdo, A. Aertsen, M. Ackermann, M. De Paepe, E. Slack, W.D. Hardt, Science (New York, N.Y.) **355**(6330) (2017) 1211.
30. C.M. Johnson, A.D. Grossman, Annual review of genetics **49** (2015) 577.
31. H. Ochman, J. Lawrence, E. Groisman, Nature **405** (2000) 299.
32. R. Medzhitov, D.S. Schneider, M.P. Soares, Science (New York, N.Y.) **335**(6071) (2012) 936.