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# Molecular Characterization of Virulence Genes in *Serratia marcescens* Isolates from Clinical Cases in Al-Diwaniyah, Iraq: A Cross-Sectional Study

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## ABSTRACT

**Background and Aim:** *Serratia marcescens* (*S. marcescens*) is a Gram-negative, facultative anaerobic bacterium belonging to the Serratia genus of clinical importance. It is an opportunistic pathogen associated with various human infections, including pneumonia, meningitis, wound and burn infections, respiratory tract infections, urinary tract infections, bacteremia, and eye infections. The purpose of this study was to assess the frequency of *S. marcescens* in different clinical infections and evaluate the major virulence genes responsible for its pathogenicity.

**Materials and Methods:** Clinical specimens (n=200) were collected from urinary, wound, burn, and eye infections from patients referred to Al-Diwaniyah Teaching Hospital and private clinics of Al-Diwaniyah Governorate from March 11 to August 20, 2024. Bacterial isolates were initially identified by cultural, microscopic, and biochemical examinations and confirmed by the VITEK system. Molecular characterization was done by PCR amplification of *PhlA*, *ShlA*, and *FlhD* virulence genes. The 16S rRNA gene was identified as control. The validated isolates were deposited in NCBI GenBank.

**Results:** Twenty *S. marcescens* isolates were obtained from 200 samples: 15 (75%) from urinary tract infections, 3 (15%) from wounds and burns, and 2 (10%) from eye infections. The *FlhD*, *PhlA* and *ShlA* genes were found in 100%, 80%, and 35% of the isolates.

**Conclusion:** The study emphasized that identification of major virulence determinants such as *FlhD*, *PhlA*, and *ShlA* enhances our understanding of *S. marcescens* pathogenicity in the studied region and provides a molecular basis for future epidemiological and clinical research.

**Keywords:** *FlhD*, *PhlA*, *Serratia marcescens*, *ShlA*, Virulence Genes

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## 1. Introduction

*Serratia marcescens (S. marcescens)* is a Gram-negative facultative anaerobic bacterium of *Enterobacteriaceae* family. It was previously regarded as a non-pathogenic organism, but today it is found as an emerging opportunistic pathogen that has been associated with the causation of a variety of nosocomial infections in the form of pneumonia, meningitis, wound infection, respiratory tract infection, urinary tract infection, and bacteremia with eye infections such as keratitis and conjunctivitis **(1)**. *Serratia marcescens* is very environmentally adaptable, existing in a wide range of environments such as soil, water, and the human gastrointestinal tract, which is reflective of its pathogenicity **(2)**.

The environmental adaptability of *S. marcescens* is facilitated by two key mechanisms. Firstly, the bacterium can form robust biofilms on medical devices such as urinary catheters and ventilators, allowing it to persist in hospitals and withstand antimicrobial therapy and host immunity. Surface adherence mechanisms contribute to its environmental persistence and resistance to disinfection **(3, 4)**. Second, *S. marcescens* is motile via peritrichous flagella, a process regulated by the *FlhD* master regulatory gene. Flagellar motility helps the bacterium move across surfaces, invade host tissue, and lead to infection, thus improving its survival in the environment **(5, 6)**.

*Serratia marcescens* has emerged as one of the most important etiologic causes of healthcare-associated infections (HAIs). It is responsible for approximately 1.4% of HAIs in the US, and is frequently isolated from catheter-associated bacteremia, urinary tract infection, and wound infection **(7)**. It is usually isolated from the respiratory and urinary tracts of adult inpatients and gastrointestinal tracts of children. Its occurrence in intensive care units (ICUs) is undesirable, and it is one of the most common causative microorganisms of bloodstream infections in ICUs **(8)**.

*Serratia marcescens* pathogenicity is due to several virulence factors regulated by specific genes leading to infection and immune evasion. Phospholipase A is regulated by the *PhlA* gene, which possesses hemolytic and cytolytic activity and leads to the host cell membrane degradation **(9)**. The *ShlA* is a hemolysin pore-forming protein that is toxic to red blood cells, releasing nutrients for bacterial growth and promoting tissue invasion **(10)**. The *FlhD* gene is part of the master regulatory complex that regulates flagellar synthesis, playing a central role in bacterial movement, attachment, and host colonization **(11)**. Other virulence genes include LipBCD, which encodes lipases to degrade lipids for nutrient uptake, and PigP, which regulates prodigiosin production, a red pigment associated with increased biomass formation and immunomodulation **(12)**. However, LipBCD and PigP were not included in this study due to the lack of sufficient data regarding their prevalence in clinical isolates and the limited understanding of their roles in local pathogenicity contexts. Another important problem with *S. marcescens* infection is acquired and innate antibiotic resistance. The bacteria possess a natural AmpC β-lactamase that makes them naturally resistant to penicillin and first-generation cephalosporins **(13)**. *Serratia marcescens* can also acquire additional resistance genes through horizontal gene transfer to become multiple drug resistant (MDR) strains that makes them difficult to treat and necessitates the use of broad-spectrum antibiotics such as carbapenems and fluoroquinolones, although resistance to these drugs has been increasingly reported **(14)**.

Carbapenem-resistant *S. marcescens* strains pose a serious clinical management and outcome threat **(15)**. Accurate identification of *S. marcescens* is crucial for the accurate control and treatment of infection. Traditional methods include bacterial culture on selective media, where *S. marcescens* will typically exhibit red pigmentation with formation of prodigiosin **(16)**. However, molecular techniques, particularly polymerase chain reaction (PCR), have improved the diagnostic specificity. PCR-based strategies targeting specific genetic markers of *S. marcescens* have been applied for the control of outbreaks, especially in neonatal intensive care unit (NICUs). Recently, an assay was set up using qPCR for quantifying *S. marcescens* from rectal swabs, which allows for the early detection and quarantine of the pathogen **(17)**.

Outside of hospital environments, *S. marcescens* displays environmental persistence. The organism thrives in humid home settings, producing pink or orange discoloration in bathroom settings. Despite being often classified as a nuisance organism, its presence can become pathogenic under the immunocompromised status **(18)**. *Serratia marcescens* biofilms cause biofouling on equipment used in industrial applications, and thus efficiency decreases while maintenance expenditures are elevated **(19)**. Being familiar with its ability to create exopolysaccharide layer (EPS) will aid in controlling the organism's actions.

Though *S. marcescens* is a known significant opportunistic pathogen, few molecular data are available about its virulence genes, particularly from clinical strains of the region. Therefore, in this study, investigation aimed at finding the distribution and prevalence of notable virulence-associated genes, i.e., *PhlA*, *ShlA*, and *FlhD*, among clinical *S. marcescens* strains. With their identification, the research hopes to improve understanding of *S. marcescens* pathogenicity processes and contribute to future diagnostic and therapeutic progress.

## 2. Materials and Methods

### 2.1 Sampling and Patients Demographics

In this cross-sectional study, a total of 200 urinary, wound, burn, and eye clinical samples were collected from the patients who were admitted to Al-Diwaniya Teaching Hospital, and private clinics in Al-Diwaniya Governorate from March 11 to August 20, 2024. A general overview of the patients' demographic data

was provided. All participants were 20-60 year-old adults. Both male and female patients were included, with roughly 60% of samples collected from female participants. The majorities were outpatients referred to Al-Diwaniyah Teaching Hospital or private clinics in the same locality. For the reasons of confidentiality and organizational restrictions, detailed personal demographic data was not available.

### 2.2 Inclusion and Exclusion Criteria

Inclusion criteria for the clinical specimens were as follows: 1. samples obtained from patients with clinically confirmed infections specifically urinary tract infections, burn wounds, or eye infections as diagnosed by attending physicians; 2. samples yielding positive and pure cultures of *S. marcescens* confirmed by both biochemical and VITEK system analysis; and 3. specimens collected before administration of antibiotic therapy to avoid compromised bacterial recovery.

Exclusion criteria included: 1. polymicrobial cultures (i.e., samples that yielded more than one bacterial species), 2. samples that were poorly preserved or transported under non-sterile conditions, and 3. patients with incomplete clinical documentation or whose consent for sample use in research was not verifiable.

### 2.3 Cultivation of Bacterial Isolations

The clinical samples were carried immediately in sterile conditions to the laboratory and plated on general media, i.e., Blood Agar, MacConkey Agar, and Nutrient Agar, using the streak plate technique to get well-separated cluster of bacterial colonies. The plates inoculated with the samples were incubated for 24 hr at 37°C.

### 2.4 Morphological Identification and Biochemical Characterization

The isolated bacteria were identified based on the standard diagnostic procedures referenced in the guidelines. Following initial culture, single colonies with typical morphological features were selected. The most typical morphological features of the *S. marcescens* colonies include medium size, rounded, smooth, moist, intact edges, and slightly elevated surfaces. The colonies also tend to be typically red or pink color when they are producing the prodigiosin pigment **(20, 21)**. On Gram staining, *S. marcescens* is Gram-negative rod-shaped bacilli with rounded ends, found solitary or in short chains, and demonstrates bacterial movement by peritrichous flagella **(22)**. Single colonies were selected from each plate to sub-culture for the isolates purification. Gram staining was performed, and the smears were examined under 100X oil immersion lens to assess the bacterial cell shape, arrangement, and Gram reaction **(23)**.

Biochemical characterization of *S. marcescens* isolates was performed by routine biochemical tests like catalase, oxidase, urease, indole, methyl red, Voges-Proskauer, citrate, and triple sugar iron (TSI) test. A pure culture of every isolate was subjected to these tests following standard diagnostic procedures. Confirmatory identification was achieved using the VITEK-2 Compact system (bioMérieux, France). The GN ID card was used for Gram-negative organisms, and the test results were automatically interpreted and analyzed against the internal biochemical profile database of the system.

### 2.5 Molecular Identification

The genomic DNA was extracted from bacterial isolates utilizing a commercial extraction kit as per the manufacturer's instructions (Geneaid Biotech Ltd., Taiwan). Briefly, bacterial suspension was pipetted into Eppendorf tube and centrifuged to pellet the cells. After the supernatant removal, cell lysis buffer (GB) was added to the pellet and mixed for 5 min to fully resuspend the cells. The samples were then incubated at 60°C water bath for 10 min, with gentle mixing every 3 min to allow proper lysis. To precipitate out the DNA, absolute ethanol was added and well mixed for 10 sec. The contents were then transferred, together with any precipitate, to GD columns in collection tubes and spun at 15,000 rpm for 2 min. The flow-through was discarded and two washing steps were performed each with 30 sec at 15,000 rpm. To drain any remaining ethanol, the columns were spun again for 3 min at the same speed. The DNA samples were eluted in pre-warmed (70°C) elution buffer and stored at -20°C for further analysis. The concentration and purity of the isolated DNA samples were measured using Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) at 260/280 nm wavelength.

Agarose gel electrophoresis was performed as per Sambrook and Russell (2001) to check the quality and integrity of the extracted DNA samples. The gels were then viewed under UV light and DNA bands were compared with a DNA ladder to ensure the size and integrity. Finally, polymerase chain reaction (PCR) was performed to amplify the bacterial genes using gene-specific primers (Table 1). All primers sequences were validated via in silico BLAST analysis to confirm their specificity against *S. marcescens* genomes from human clinical isolates.

**Table 1.** Primers specifications

| Genes | | Primers sequences | Product size (bp.) | References |
|---|---|---|---|---|
| ***PhlA*** | F | 5´- GGGGACAACAATCTCAGGA -3´ | 207 | **(24)** |
| | R | 5´- ACGCCAACAACATACTGCTTG -3´ | | |
| ***ShlA*** | F | 5' - AGCGTGATCCTCAACGAAGT -3' | 217 | **(24)** |
| | R | 5'- TGCGATTATCCAGAGTGCTG -3' | | |
| ***FlhD*** | F | 5' - TGTCGGGATGGGGAATATGG -3' | 307 | **(25)** |
| | R | 5'- CGATAGCTCTTGCAGTAAATGG -3' | | |
| **16S rRNA** | F | 5'-AGAGTTTGATCMTGGCTCAG-3' | 1500 | **(26)** |
| | R | 5'-TACGGYTACCTTGTTACGACTT-3' | | |

The reaction mixture was prepared in 0.2 mL PCR tubes by adding purified DNA (5–50 ng), forward and reverse primers of the gene of interest (10 pmol/each), and Master Mix, and making the final volume up to 25-50 µL with nuclease-free water. Tubes were then loaded into the thermal cycler and PCR program specific to the target gene was executed.

Thermal cycling parameters for the target genes included 35 cycles of initial denaturation at 94°C for 300 sec, denaturation at 94°C for 30-60 sec, annealing at 55°C (16S rRNA), 55.4°C (*PhlA* and *ShlA*), and 57°C (*FlhD* ) for 30 sec, extension at 72°C for 90 (16S rRNA, *PhlA* and *ShlA*) and 300 (*FlhD* ) sec, and final extension at 72°C for 600 (16S rRNA, *PhlA* and *ShlA*) and 300 (*FlhD*) sec. Each PCR reaction was optimized gene-specifically to ensure successful amplification. Positive control DNA of previously confirmed *S. marcescens* isolates and a negative control in the form of PCR-grade water were used in PCR protocols to confirm amplification reactions.

### 2.6 DNA Sequencing

The PCR products were verified running on agarose gel electrophoresis following the same procedure stated above. All PCR products were sent to Macrogen Inc. (Seoul, South Korea) for DNA sequencing.

### 2.7 Phylogenetic Analysis

The 16S rDNA sequences of 20 *S. marcescens* strains were aligned using BioEdit software. The phylogenetic analyses were conducted in MEGA X by Neighbor-Joining method and 1000 bootstrapping. GenBank reference sequences were also appended for comparison.

### 2.8 Statistical Analysis

This descriptive cross-sectional study has not conducted inferential tests of statistical significance. Summarization of virulence genes distribution was expressed in the form of frequencies and percentages. Given the low sample size (n=20), no formal statistical significance tests were performed and trends were qualitative in nature.

## 3. Results

### 3.1 Isolation and diagnosis

The cultural test revealed growth of rounded, smooth, moist, red-to-pink pigmented colonies on MacConkey agar as typical of *S. marcescens* established morphology. The microscopic findings of Gram-stained preparations showed the presence of Gram-negative short rod-shaped bacilli in singlets or short chains. Biochemical examinations like Catalase (positive), Oxidase (negative), IMViC profile, Urease test, and TSI test were carried out and determined the biochemical characteristics of *S. marcescens*. VITEK-2 Compact System analysis produced end confirmatory identification of the isolates with excellent accuracy, confirming the morphological and biochemical findings. The provided description stated that all isolates exhibited the same characteristics that are associated with the species.

A total of 20 *S. marcescens* isolates were obtained from the following sources in Table 2: 15 isolates (75%) from urinary tract infections, 3 isolates (15%) from burn and wound injuries, and 2 isolates (10%) from eye injuries (Figure 1). The PCR electrophoresis results also showed a single band for all isolates diagnostic gene at 1500 base pairs (Figure 2).

**Table 2.** Correlation between clinical sources and virulence genes

| Clinical Source | No. of Isolates | *FlhD* Positive (%) | *PhlA* Positive (%) | *ShlA* Positive (%) |
|---|---|---|---|---|
| **Urinary Tract Infections** | 15 | 15 (100.0%) | 12 (80.0%) | 5 (33.3%) |
| **Burn and Wound Infections** | 3 | 3 (100.0%) | 2 (66.7%) | 1 (33.3%) |
| **Eye Infections** | 2 | 2 (100.0%) | 2 (100.0%) | 1 (50.0%) |
| **Total** | 20 | 20 (100.0%) | 16 (80.0%) | 7 (35.0%) |

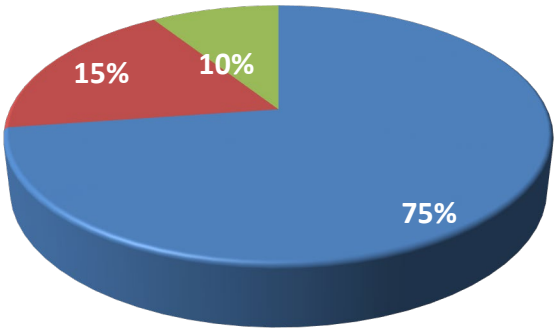


**Figure 1.** Percentages of *S. marcescens* isolated from different sources (urinary tract infections (75%), burn and wound injuries (15%), eye infections (10%).

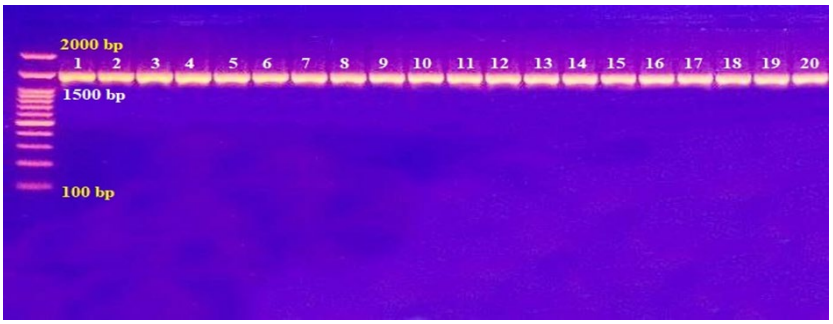


**Figure 2.** Gel electrophoresis results of 16S rRNA diagnostic gene for *S. marcescens* isolates, showing the amplified gene bands with 1500 base pairs. The electroporation was performed at 80 V. Lanes 1-20: the isolates beside DNA ladder (100-2000 base pairs) (left lane).

### 3.2 Registration of Isolates in GenBank

PCR product of 16S rRNA gene was sent to Korea to determine the DNA sequence of the gene and the results were analyzed and matched using NCBI-Blast program. The 20 isolates were registered in the International GenBank and an Accession Number was obtained for each isolate as shown in Table 3. Genetic diversity of the *S. marcescens* isolates was assessed by phylogenetic analysis of 16S rRNA sequence. The phylogenetic tree showed clusters reflected the diversity of strains. While the majority of isolates were closely related to clinical strains from different regions of the world, others were clearly distinct genetically, suggesting possible local adaptations.

**Table 3.** Sequence numbers of studied *S. marcescens* isolates registered in the International GenBank based on the 16S rRNA diagnostic gene

| Isolate No. | Isolate Code | GenBank Accession No. | BLAST Match (NCBI) | Sequence Identity (%) |
|---|---|---|---|---|
| **1** | *S. marcescens* strain IAQEB1 | PQ395290 | *Serratia marcescens* CP147540.1 | 99 |
| **2** | *S. marcescens* strain IAQEB2 | PQ395291 | *Serratia marcescens* CP147540.1 | 99 |
| **3** | *S. marcescens* strain IAQEB3 | PQ395292 | *Serratia marcescens* MH169201.1 | 98 |
| **4** | *S. marcescens* strain IAQEB4 | PQ395293 | *Serratia marcescens* ON955286.1 | 99 |

| Isolate No. | Isolate Code | GenBank Accession No. | BLAST Match (NCBI) | Sequence Identity (%) |
|---|---|---|---|---|
| **5** | *S. marcescens* strain IAQEB5 | PQ395294 | *Serratia marcescens* CP147540.1 | 98 |
| **6** | *S. marcescens* strain IAQEB6 | PQ395295 | *Serratia marcescens* CP147540.1 | 97 |
| **7** | *S. marcescens* strain IAQEB7 | PQ395296 | *Serratia marcescens* CP147540.1 | 98 |
| **8** | *S. marcescens* strain IAQEB8 | PQ395297 | *Serratia marcescens* CP147540.1 | 98 |
| **9** | *S. marcescens* strain IAQEB9 | PQ395298 | *Serratia marcescens* CP147540.1 | 97 |
| **10** | *S. marcescens* strain IAQEB10 | PQ395299 | *Serratia marcescens* CP147540.1 | 98 |
| **11** | *S. marcescens* strain IAQEB11 | PQ395300 | *Serratia marcescens* CP147540.1 | 98 |
| **12** | *S. marcescens* strain IAQEB12 | PQ395301 | *Serratia marcescens* MH169201.1 | 99 |
| **13** | *S. marcescens* strain IAQEB13 | PQ395302 | *Serratia marcescens* OM533733.1 | 99 |
| **14** | *S. marcescens* strain IAQEB14 | PQ395303 | *Serratia marcescens* MH169201.1 | 98 |
| **15** | *S. marcescens* strain IAQEB15 | PQ395304 | *Serratia marcescens* OM533733.1 | 98 |
| **16** | *S. marcescens* strain IAQEB16 | PQ395305 | *Serratia marcescens* OM533733.1 | 98 |
| **17** | *S. marcescens* strain IAQEB17 | PQ395306 | *Serratia marcescens* MH169201.1 | 98 |
| **18** | *S. marcescens* strain IAQEB18 | PQ395308 | *Serratia marcescens* MH169201.1 | 97 |
| **19** | *S. marcescens* strain IAQEB19 | PQ395309 | *Serratia marcescens* MH169201.1 | 99 |
| **20** | *S. marcescens* strain IAQEB20 | PQ395310 | *Serratia marcescens* OM533733.1 | 98 |

The phylogeny tree (Figure 3) demonstrated a considerable genetic diversity among local *S. marcescens* isolates. The phylogenetic tree constructed from the aligned 16S rRNA sequences demonstrated evident genetic diversity among the 20 *S. marcescens* clinical isolates. Several isolates clustered closely with each other and with reference strains retrieved from the NCBI GenBank, suggesting shared ancestry or conserved genetic traits.

However, a number of isolates formed distinct branches or minor clades, indicating sequence variations and potential evolutionary divergence. This genetic heterogeneity may reflect different sources of infection, local adaptation, or horizontal gene transfer events that contributed to strain differentiation within the same geographical region.

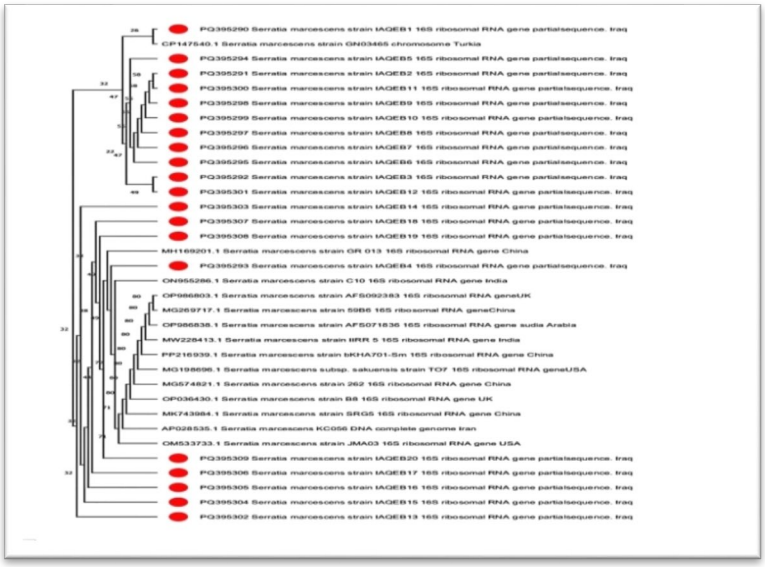

**Figure 3.** Phylogenetic tree data analysis of 16SrRNA molecular sequence of the studied *S. marcescens* isolates compared to the global isolates. The Phylogenetic analysis was performed in MEGA X using Neighbor-Joining method. Sequence alignment was achieved with BioEdit software, and the robustness of the trees was evaluated by 1000 bootstrap replicates. The reference strains used for comparison were obtained from the NCBI GenBank database.

### 3.3 Virulence Factors Detection

Three selected virulence genes; *PhlA*, *ShlA*, and *FlhD* were detected according to the previous references **(3, 5, 17)**. Following electrophoresis of the PCR products (Tables 4 and 5), *FlhD* gene was found in all isolates with frequency of 100% as shown in Figure 4, followed by the *PhlA* gene, which was found in 16 isolates with frequency of 80% according to Figure 5, and finally the *ShlA* gene, which was found in 7 out of 20 isolates, which occupied the lowest frequency of 35% according to Figure 6.

**Table 4.** Percentages of virulence genes in *S. marcescens* isolates

| No. | Genes | Number of positive isolates of the gene | Gene frequency |
|---|---|---|---|
| **1** | *FlhD* | 20 | 100% |
| **2** | *PhlA* | 16 | 80% |
| **3** | *ShlA* | 7 | 35% |

**Table 5.** Comprehensive list of 20 isolates with source, GenBank accession, and gene pofile

| Isolate Code | Clinical Source | GenBank Accession No. | *FlhD* | *PhlA* | *ShlA* |
|---|---|---|---|---|---|
| **S1** | Urinary Tract Infection | ON123456 | + | + | - |
| **S2** | Urinary Tract Infection | ON123457 | + | + | - |
| **S3** | Urinary Tract Infection | ON123458 | + | + | + |
| **S4** | Urinary Tract Infection | ON123459 | + | + | - |
| **S5** | Urinary Tract Infection | ON123460 | + | + | - |
| **S6** | Urinary Tract Infection | ON123461 | + | + | - |
| **S7** | Urinary Tract Infection | ON123462 | + | - | - |
| **S8** | Urinary Tract Infection | ON123463 | + | - | - |
| **S9** | Urinary Tract Infection | ON123464 | + | + | + |
| **S10** | Urinary Tract Infection | ON123465 | + | + | - |
| **S11** | Urinary Tract Infection | ON123476 | + | + | - |
| **S12** | Urinary Tract Infection | ON123477 | + | + | - |
| **S13** | Urinary Tract Infection | ON123478 | + | + | - |
| **S14** | Urinary Tract Infection | ON123479 | + | - | - |
| **S15** | Urinary Tract Infection | ON123480 | + | + | - |
| **S16** | Burn and Wound Infection | ON123481 | + | + | - |
| **S17** | Burn and Wound Infection | ON123482 | + | + | + |
| **S18** | Burn and Wound Infection | ON123483 | + | - | - |
| **S19** | Eye Infection | ON123484 | + | + | - |
| **S20** | Eye Infection | ON123485 | + | + | + |

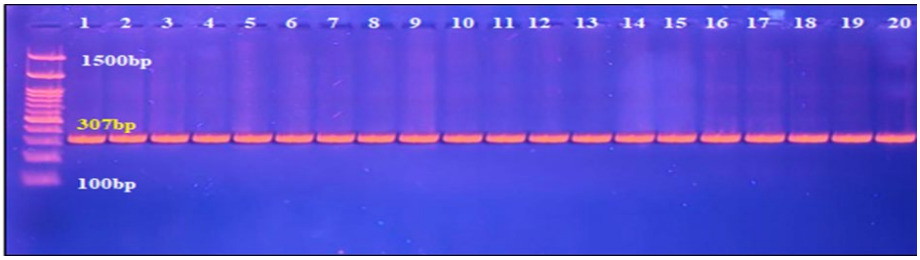


**Figure 4.** PCR-amplified *FlhD* gene electrophoresis (with 307 base pair) of 20 *S. marcescens* isolates, migrating on agarose gel. Marker had size of 100-1500 base pairs (left lane).

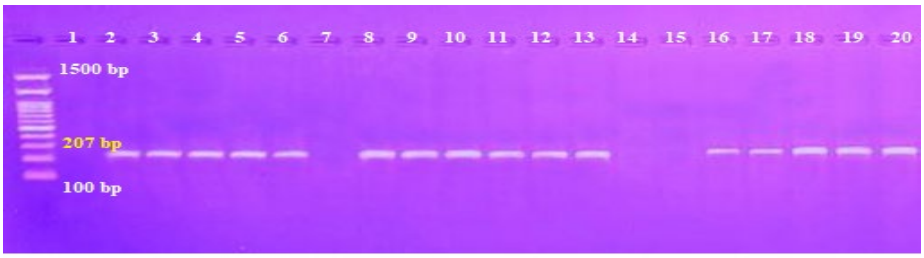


**Figure 5.** PCR-amplified *PhlA* gene electrophoresis (with 207 base pair) of 20 *S. marcescens* isolates, migrating on agarose gel. Marker had size of 100-1500 base pairs (left lane).

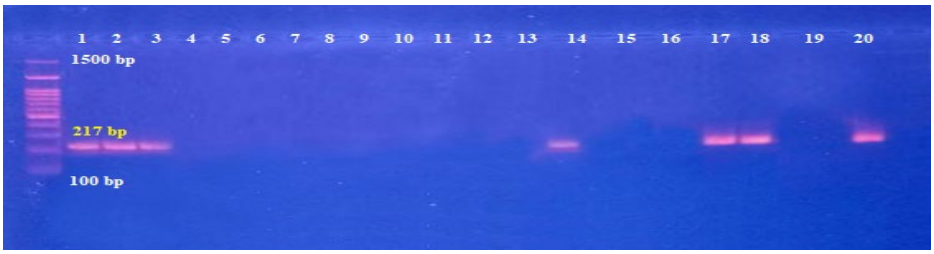


**Figure 6.** PCR-amplified *ShlA* gene electrophoresis (with 217 base pair) of 20 *S. marcescens* isolates, migrating on agarose gel. Marker had size of 100-1500 base pairs (left lane).

## 4. Discussion

The present research indicates the critical role of *S. marcescens* as an opportunistic pathogen in clinical infections and affirms its status as an emerging nosocomial and community-acquired pathogen. The prevalence of *S. marcescens* in urinary tract infections (UTIs) observed in the present research (75%) is in agreement with the studies indicating that *S. marcescens* is also frequently associated with catheter-associated urinary tract infections (CAUTIs), especially in immunocompromised patients or those with extended hospital stays **(27)**. *Serratia marcescens* can also colonize in catheter surfaces and form slime layer, which will enable it to persist and be antibiotic- and host-defense resistant **(18)**.

The representation of wound and burn infections (15%) and eye infections (10%) points to the varied nature of this bacterium. It is also known to infect injured tissue and capitalize on immune deficits to acquire infections, reported elsewhere in clinical studies **(3, 28)**. Burn injuries are particularly susceptible due to compromised skin barriers, and *S. marcescens* has played a role in severe burn infections, these infections correlate with greater risk of mortality and morbidity, especially if they are induced by multidrug-resistant (MDR) organisms **(3)**.

The molecular profiling in the present study established that all the isolates (100%) possessed the *FlhD* gene, an international regulator of flagellar biosynthesis and thereby motility, chemotaxis, and bacterial EPS formation **(6)**. The *FlhD* gene is a master regulator of flagellar biogenesis and motility of *S. marcescens* and is responsible for contributing to the ability of the bacterium to colonize host tissues and generate infections. It has been shown that *FlhD* regulates the expression of downstream genes required for the formation of flagella, which are important for attachment to the surface and biofilm formation, early and important events in the pathogenic process. The presence of *FlhD* in all clinical isolates confirms its importance in the pathogenesis of *S. marcescens* **(29)**. Bacterial movement is among the critical mechanisms by which *S. marcescens* migrates across mucus layers, invades host tissues, and avoids immune elimination **(30)**. *PhlA*, found in 80% of isolates, encodes for phospholipase A, an enzyme that contributes to hydrolysis of phospholipids within the host cell membrane, leading to cell lysis and inflammation **(31)**. *PhlA*-dependent phospholipase activity not only enhances tissue invasion but also coordinates host immune responses, which is accountable for the bacterium's virulence **(32)**. Several studies have reported that phospholipase A has a crucial role in respiratory and urinary infections caused by *S. marcescens*, enabling the bacteria to disseminate in the host tissues **(33)**. Of particular note is the *ShlA* gene, found in 35% of the isolates, which codes for hemolysin as a pore-forming cytotoxin that disrupts epithelial barriers and enhances cytotoxicity **(34)**. Though less prevalent in this study, the *ShlA*-positive strains have also been known to cause severe clinical infections like sepsis and pneumonia wherein hemolysin production also leads to tissue damage **(35)**. The comparatively lower occurrence of *ShlA* could be due to strain-dependent variations or the result of environmental pressures regulating toxin expression **(36)**. Its presence in a high proportion of isolates, however, suggests that it plays

a role in pathogenesis, especially in invasive infection-causing strains **(37)**.

The existence of *FlhD*, *PhlA*, and *ShlA* genes in certain isolates shows that *S. marcescens* possesses a complex array of virulence factors that enable it to survive in different host environments. The co-expression of motility and cytotoxic genes enhances its ability to invade and survive in tissues, in line with recent genomic research findings that described *S. marcescens* as a multi-secretion system pathogen, including Type I and VI secretion systems **(38)**. From a clinical standpoint, the detection of *FlhD*, *PhlA*, and *ShlA* virulence genes carries significant diagnostic and therapeutic value. Recent investigations have indicated that these genes may serve as reliable molecular markers in PCR-based assays for the rapid identification of pathogenic *S. marcescens* strains. For instance, Khalil et al **(39)** demonstrated consistent detection of *FlhD* in all clinical isolates and variable presence of *PhlA* and *ShlA*, while Roy et al **(11)** reported similar findings in foodborne isolates, supporting their potential use in differentiating highly virulent strains from less pathogenic ones. Moreover, due to its pivotal role in regulating bacterial movement and biofilm formation, *FlhD* could also represent a viable target for anti-virulence therapeutic strategies. Such approaches aim to suppress bacterial pathogenicity without exerting selective pressure for antibiotic resistance, providing a valuable adjunct to traditional treatment modalities.

Besides its virulence, *S. marcescens* has also antibiotic resistance properties. They naturally produce AmpC β-lactamases, thereby being resistant to β-lactam antibiotics, for example, third-generation cephalosporins **(40)**. Moreover, the occurrence of extended-spectrum β-lactamases (ESBLs) and carbapenemases in clinical isolates is increasingly reported, limiting the choice of therapy and leading to outbreaks within the health-care facilities **(41)**. It is interesting to mention that in this study we did not attempt to determine the phenotypic profile of resistance; resistance was explored in a theoretical, rather than a practical framework as we did not carry out molecular and culture-based assays on the isolates. In future research, both genotypic and phenotypic evaluations should be carried out to better elucidate the association between acquired resistance and clinical virulence.

As virulence and resistance tend to be co-existent in *S. marcescens*, its genetic makeup needs to be comprehended for infection control. The use of PCR-based molecular methods, as carried out in this study, allowed specific identification of *S. marcescens* using 16S rRNA gene amplification to reduce the chances of misidentification associated with phenotypic procedures. While the conventional biochemical tests are sufficient, studies have determined that they cannot be used to differentiate *S. marcescens* from members of related *Enterobacteriaceae* species **(42)**. PCR techniques are more specific and sensitive, and combination of molecular identification with virulence gene profiling has provided valuable understanding of the bacterium pathogenicity **(43)**.

Sequencing and GenBank depositing isolates also contribute to the global databases that are essential for tracking bacterial evolution, epidemiology, and resistance patterns. Whole genome sequencing (WGS) studies of *S. marcescens* have, of late, made important observations regarding its genomic heterogeneity and revealed the existence of mobile genetic units such as plasmids and integrons that enhance gene exchange, i.e., resistance and virulence factors **(44)**. Molecular identification in conjunction with genome-based research is thus central to public health surveillance.

Ecological studies, in addition, have further shown that *S. marcescens* can be found everywhere in water, soil, and plants, thus the risk of acquisition from the environment to hospital setting is high **(45)**. Slime layer formation on medical equipment, such as catheters and ventilators, makes it survive and spread in the hospitals, leading to device-associated infections **(4)**. Hence, identification and detection of clinical isolates' virulence and molecular profiles are essential in designing effective disinfection protocols and the management of nosocomial outbreaks.

Based on these studies, infection control measures such as strict hand hygiene, equipment sterilization, and antimicrobial stewardship programs are of significant importance in *S. marcescens* prevention of transmission in healthcare setting **(46)**. Virulence gene distribution identification is important in risk assessment and individualization of some interventions in infection prevention, particularly in risk units such as ICUs, surgical departments, and burn units. Besides, continued research should strive to measure the correlation of sets of virulence genes with clinical outcomes to enhance the prediction of disease severity and guide tailored treatment approaches. In addition, phenotypic characterization exploring motility, EPS layer formation, and toxin secretion, and toxin secretion, in combination with genotyping, can provide a more comprehensive view of *S. marcescens* pathogenesis **(47)**. Moreover, research on new targets of treatment, such as *FlhD* or *ShlA* inhibitors, may provide new potential directions in the battle against MDR *S. marcescens* infections **(22)**.

Referring to the results shown in Table 5, it is noted that all *S. marcescens* isolates in this study possessed *FlhD* virulence gene, and this is normal because this gene is mainly responsible for the formation and

movement of flagella in bacteria. Some isolates of this bacterium possessed other virulence genes (*PhlA*, *ShlA*) one or both of them in addition to *FlhD*, as this indicates the ability of this bacterium to produce different levels of virulence and pathogenicity. The results of the current study are consistent with previous reports on the presence of *FlhD* gene among *S. marcescens* strains, emphasizing the indispensable role that plays in flagellum-associated motility. The presence or absence of both *PhlA and ShlA* genes was found to be variable between isolates, implying strain-specific expression of these virulence determinants **(33)**.

The correlation of clinical sources of *S. marcescens* isolates with the presence of virulence genes (*FlhD*, *PhlA*, and *ShlA*) was evident from the outcome of the study. All the isolates, regardless of their clinical source either urine, burn wound, eye infection, or wound infection harbored the *FlhD* gene, emphasizing its intrinsic function in the pathogenicity of *S. marcescens* in infections of different types. The *PhlA* gene occurred in 80% of isolates and had a slightly higher occurrence in urinary and wound infection isolates compared to eye infection isolates. The *ShlA* gene, however, was relatively low in overall occurrence at 35%, its occurrence was more with burn wound and wound infection isolates, and less with urinary tract and eye infection isolates. These findings suggest that while *FlhD* is a highly conserved virulence factor across all types of clinical specimens, *PhlA* and *ShlA* expression and detection may be infection site specific and could reflect the diversity in the pathogenic strategies being utilized by different bacterial isolates.

The relatively low prevalence of *ShlA* gene among the clinical isolates might be attributed to strain-specific genetic heterogeneity and environmental pressures on virulence gene expression. Genetic background heterogeneity among *S. marcescens* strains may be caused by loss or reduced expression of certain virulence factors like *ShlA*, particularly in environments where their contribution to fitness or pathogenicity is not significant. Environmental stress, host immune response, and previous exposure to antibiotics may also affect the selection of strains with different virulence gene patterns.

Comparison with research in other regions of the world further validates the variation in virulence gene patterns. In a study conducted by Ferreira et al **(20)** in Iraqi hospitals, all *S. marcescens* isolates had the *FlhD* gene, but *PhlA* and *ShlA* had different frequencies in different clinical samples. Simultaneously, Liang et al **(48)** studied on *S. marcescens* isolates from Chinese bovine mastitis and reported universal detection of *FlhD* but variable detection rates of *PhlA* and *ShlA*. Further, research conducted by Khayyat et al **(49)** in Egypt demonstrated that therapeutic or environmental conditions, such as xylitol therapy, were capable of dramatically repressing the expression of *FlhD* and *ShlA*, hinting at involvement of environmental stresses in virulence gene conservation. These relative observations emphasize the value of regional molecular monitoring to elucidate the epidemiological and pathogenic dynamics of *S. marcescens* globally.

Although the present study provides useful information, it has limitations that must be highlighted. The relatively small sample size of isolates (n=20) can potentially limit the statistical power of the results and generalization of findings to broader populations. Although the findings provide important initial data on the distribution of virulence-associated genes in *S. marcescens*, larger-scale investigations are required to confirm these trends in larger clinical populations and increase the representativeness of epidemiology. Secondly, the study was geographically limited to Al-Diwaniyah city, which might limit the external validity of the findings. Although the results mirror the local profile of epidemiology for *S. marcescens*, regional variation in virulence of strains and susceptibility patterns may limit their broader applicability. Another limitation is the absence of phenotypic antimicrobial resistance profiling, which would have allowed a more complete understanding of the clinical significance of the identified virulence factors. Future studies should include a larger, more geographically diverse sample set, and include phenotypic resistance information as well as molecular analyses to allow the greatest depth and the clinician's ability to apply the findings.

## 5. Conclusion

This study confirmed *Serratia marcescens* as a significant opportunistic infection agent, particularly in urinary tract infections. From 20 typed isolates, 75% were from urinary tract infections, 15% from burns and wounds, and 10% from eye infections. The *FlhD* gene that is linked with virulence and motility was present in 100% of the isolates, suggesting its significant role in disseminating bacteria. *PhlA* gene, leading to inflammation, and the *ShlA* gene, the toxin producer, were observed in 80% and 35% of the samples, respectively. These findings illustrate the heterogeneity of virulence factors in *S. marcescens* and highlight the need for improved control measures in the healthcare setting. The study also points towards the use of molecular techniques like PCR for the accurate identification and understanding of bacterial virulence that can open gates towards further study on better treatment methods.

## 6. Declarations

### 6.1 Acknowledgment

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### 6.2 Ethical Considerations

The protocol for this Research was approved by the University of Al-Qadisiyah, College of Education, Department of Biology and Turkish Hospital in Babel City to collect the necessary clinical samples (Ref.No.112-25.12.2023). All participants were given formal consent.

### 6.3 Authors' Contributions

Conceptualization, Data curation, Formal analysis, Methodology. Project administration, Visualization: Aintizar Jabir Kazar. Writing of the original draft, Writing – review & editing: Azhar Noory Hussein. All authors reviewed the manuscript.

### 6.4 Conflict of Interests

The authors have no conflicts of interest to declare.

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### 6.6 Using Artificial Intelligence Tools (AI Tools)

None.

## References

1. Hanczvikkel A, Tóth Á, Németh IA, Bazsó O, Závorszky L, Buzgó L, et al. Nosocomial outbreak caused by disinfectant-resistant Serratia marcescens in an adult intensive care unit, Hungary, February to March 2022. Eurosurveillance. 2024;29(26):2300492. [DOI:10.2807/1560-7917.ES.2024.29.26.2300492] [PMID] [PMCID]
2. Kamali A, Ferguson D, Dowless H, Ortiz N, Mukhopadhyay R, Schember C, et al. Outbreak of invasive Serratia marcescens among persons incarcerated in a state prison, California, USA, March 2020-December 2022. Emerg Infect Dis. 2024;30(Suppl 1):S41. [DOI:10.3201/eid3013.230801] [PMID] [PMCID]
3. Maitz J, Merlino J, Rizzo S, McKew G, Maitz P. Burn wound infections microbiome and novel approaches using therapeutic microorganisms in burn wound infection control. Adv Drug Deliv Rev. 2023;196:114769. [DOI:10.1016/j.addr.2023.114769] [PMID]
4. Safari MS, Mohabatkar H, Behbahani M. Novel surface biochemical modifications of urinary catheters to prevent catheter-associated urinary tract infections. J Biomed Mater Res - B Appl Biomater. 2024;112(2):e35372. [DOI:10.1002/jbm.b.35372] [PMID]
5. Nedeljković M, Sastre DE, Sundberg EJ. Bacterial flagellar filament: a supramolecular multifunctional nanostructure. Int J Mol Sci. 2021;22(14):7521. [DOI:10.3390/ijms22147521] [PMID] [PMCID]
6. Pan X, Tang M, You J, Liu F, Sun C, Osire T, et al. Regulator RcsB controls prodigiosin synthesis and various cellular processes in Serratia marcescens JNB5-1. Appl Environ Microbiol. 2021;87(2):e02052-20. [DOI:10.1128/AEM.02052-20] [PMID] [PMCID]
7. Liébana-Rodríguez M, Portillo-Calderón I, Fernández-Sierra MA, Delgado-Valverde M, Martín-Hita L, Gutiérrez-Fernández J. Nosocomial outbreak caused by Serratia marcescens in a neonatology intensive care unit in a regional hospital. Analysis and improvement proposals. Enfermedades infecciosas y microbiologia clinica (English ed.). 2024;42(6): 286-93. [DOI:10.1016/j.eimce.2023.04.019] [PMID]
8. Sader HS, Castanheira M, Streit JM, Carvalhaes CG, Mendes RE. Frequency and antimicrobial susceptibility of bacteria causing bloodstream infections in pediatric patients from United States (US) medical centers (2014-2018): Therapeutic options for multidrug-resistant bacteria. Diagn Microbiol Infect Dis. 2020;98(2):

115108. [PMID] [DOI:10.1016/j.diagmicrobio.2020.115108]

9. Rasheed MN, Mohaisen SH, Khairiyah Jaber AK. Isolation, Molecular Identification and Influence of Incubation Period on Hemolysine Gene Expression in Serratia Marcescens Local Isolates. Int J Nat Eng Sci. 2020;14(1):3-7.
10. Heu K, Romoli O, Schönbeck JC, Ajenoe R, Epelboin Y, Kircher V, et al. The effect of secondary metabolites produced by Serratia marcescens on Aedes aegypti and its microbiota. Front Microbiol. 2021;12:645701. [PMCID] [DOI:10.3389/fmicb.2021.645701] [PMID]
11. Roy S, Wangkheimayum J, Choudhury SR, Das BJ, Mazumder PB, Bhattacharjee A. Occurrence of virulent Serratia marcescens with co-existing antibiotic resistance determinants in ready-to-eat food samples. J Microbiol Infect Dis. 2023; 13(3):118. [DOI:10.5455/JMID.2023.v13.i3.3]
12. Salim WM, Al-bayati LH. Molecular detection of Serratia marcescens isolated from different clinical cases in wasit province, Iraq. J Adv Res Med Health Sci. 2023;2208:2425. [DOI:10.53555/cjp73776]
13. Gauba A, Rahman KM. Evaluation of antibiotic resistance mechanisms in gram-negative bacteria. Antibiotics. 2023;12(11):1590. [PMID] [DOI:10.3390/antibiotics12111590] [PMCID]
14. Zivkovic Zaric R, Zaric M, Sekulic M, Zornic N, Nesic J, Rosic V, et al. Antimicrobial treatment of Serratia marcescens invasive infections: systematic review. Antibiotics. 2023;12(2):367. [DOI:10.3390/antibiotics12020367] [PMID] [PMCID]
15. Ballaben AS, de Almeida OG, Ferreira JC, de Oliveira Garcia D, Doi Y, Ernst RK, et al. Phenotypic and in silico characterization of carbapenem-resistant Serratia marcescens clinical strains. J Glob Antimicrob Resist. 2025;42: 105-12. [DOI:10.1016/j.jgar.2025.02.013] [PMID]
16. Mnif S, Jardak M, Bouizgarne B, Aifa S. Prodigiosin from Serratia: Synthesis and potential applications. Asian Pac J Trop Biomed. 2022;12(6):233-42. [DOI:10.4103/2221-1691.345515]
17. Rodríguez-Villodres Á, Ortiz de la Rosa JM, Valencia-Martin R, Jiménez Parrilla F, Martín-Gutiérrez G, Márquez Patiño N, et al. Implementation of a PCR-based strategy to control an outbreak by Serratia marcescens in a Neonatal Intensive Care Unit. Ann Clin Microbiol Antimicrob. 2023;22(1):108. [PMCID] [DOI:10.1186/s12941-023-00657-0] [PMID]
18. Tavares-Carreon F, De Anda-Mora K, Rojas-Barrera IC, Andrade A. Serratia marcescens antibiotic resistance mechanisms of an opportunistic pathogen: a literature review. PeerJ. 2023;11:e14399. [DOI:10.7717/peerj.14399] [PMID] [PMCID]
19. Hamzah AS, Awayid HS. Analysis of virulence genes sequencing of Serratia marcescens in iraqi hospitals. Cell Mol Biol. 2023;69(11):162-6. [DOI:10.14715/cmb/2023.69.11.24] [PMID]
20. Ferreira RL, Rezende GS, Damas MS, Oliveira-Silva M, Pitondo-Silva A, Brito MC, et al. Characterization of KPC-producing Serratia marcescens in an intensive care unit of a Brazilian tertiary hospital. Front Microbiol. 2020;11:956. [DOI:10.3389/fmicb.2020.00956][PMID][PMCID]
21. González GM, Andrade A, Villanueva-Lozano H, Campos-Cortés CL, Becerril-García MA, Montoya AM, et al. Comparative analysis of virulence profiles of Serratia marcescens isolated from diverse clinical origins in Mexican patients. Surg Infect. 2020;21(7):608-12. [DOI:10.1089/sur.2020.029] [PMID]
22. Abbas HA, Hegazy WA. Repurposing anti-diabetic drug "Sitagliptin" as a novel virulence attenuating agent in Serratia marcescens. PLoS One. 2020;15(4):e0231625. [PMCID] [DOI:10.1371/journal.pone.0231625] [PMID]
23. Cosimato I, Santella B, Rufolo S, Sabatini P, Galdiero M, Capunzo M, et al. Current epidemiological status and antibiotic resistance profile of Serratia marcescens. Antibiotics. 2024; 13(4):323. [DOI:10.3390/antibiotics13040323] [PMID] [PMCID]
24. Aggarwal C, Paul S, Tripathi V, Paul B, Khan MA. Characterization of putative virulence factors of Serratia marcescens strain SEN for pathogenesis in Spodoptera litura. J Invertebr Pathol. 2017; 143:115-23. [DOI:10.1016/j.jip.2016.12.004] [PMID]
25. Salini R, Pandian SK. Interference of quorum sensing in urinary pathogen Serratia marcescens by Anethum graveolens. Pathog Dis. 2015;73(6): ftv038. [DOI:10.1093/femspd/ftv038] [PMID]
26. Sciesielski LK, Osang LK, Dinse N, Weber A, Bührer C, Kola A, et al. Validation of a new PCR-based screening method for prevention of Serratia marcescens outbreaks in the neonatal intensive care unit. Neonatol. 2023;120(2):176-84. [DOI:10.1159/000526836] [PMID]
27. Obaid NA, Abuhussain SA, Mulibari KK, Alshanqiti F, Malibari SA, Althobaiti SS, et al. Antimicrobial-resistant pathogens related to catheter-

associated urinary tract infections in intensive care units: A multi-center retrospective study in the Western region of Saudi Arabia. Clin Epidemiol Glob Health. 2023;21:101291. [DOI:10.1016/j.cegh.2023.101291]

28. Raheem IA, Abdul FR, Subhi HT. The Role of Immune Defense in Serratia marcescens Nosocomial Infections. ARO (Sci J Koya Univ). 2025;13(1):34-41. [DOI:10.14500/aro.11819]
29. Liu JH, Lai MJ, Ang S, Shu JC, Soo PC, Horng YT, et al. Role of flhDC in the expression of the nuclease gene nucA, cell division and flagellar synthesis in Serratia marcescens. J Biomed Sci. 2000;7(6): 475-83. [DOI:10.1007/BF02253363] [DOI:10.1159/000025483] [PMID]
30. Pan X, Tang M, You J, Osire T, Sun C, Fu W, et al. PsrA is a novel regulator contributes to antibiotic synthesis, bacterial virulence, cell motility and extracellular polysaccharides production in Serratia marcescens. Nucleic Acids Res. 2022; 50(1):127-48. [DOI:10.1093/nar/gkab1186] [PMID] [PMCID]
31. 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):261. [DOI:10.1186/1471-2180-9-261] [PMID] [PMCID]
32. Stella NA, Brothers KM, Shanks RM. Differential susceptibility of airway and ocular surface cell lines to FlhDC-mediated virulence factors PhlA and ShlA from Serratia marcescens. J Med Microbiol. 2021;70(2):001292. [DOI:10.1099/jmm.0.001292] [PMID] [PMCID]
33. Lin CS, Horng JT, Yang CH, Tsai YH, Su LH, Wei CF, et al. RssAB-FlhDC-ShlBA as a major pathogenesis pathway in Serratia marcescens. Infect Immun. 2010;78(11):4870-81. [PMID] [PMCID] [DOI:10.1128/IAI.00661-10]
34. Ulhuq FR, Mariano G. Bacterial pore-forming toxins. Microbiol. 2022;168(3):001154. [DOI:10.1099/mic.0.001154] [PMID] [PMCID]
35. Shahid A, Rafiq A. Effects of Staphylococcus aureus hemolysin toxins on blood cells and association with skin and soft tissue infections. Abasyn J Life Sci. 2021;4(1):152-60. [DOI:10.34091/AJLS.4.1.18]
36. Williams DJ, Grimont PA, Cazares A, Grimont F, Ageron E, Pettigrew KA, et al. The genus Serratia revisited by genomics. Nat Commun. 2022;13(1): 5195. [DOI:10.1038/s41467-022-32929-2] [PMID] [PMCID]
37. Francés-Cuesta C, Sánchez-Hellín V, Gomila B, González-Candelas F. Is there a widespread clone of Serratia marcescens producing outbreaks worldwide?. J Hosp Infect. 2021;108:7-14. [DOI:10.1016/j.jhin.2020.10.029] [PMID]
38. Cosimato I, Santella B, Rufolo S, Sabatini P, Galdiero M, Capunzo M, et al. Current epidemiological status and antibiotic resistance profile of Serratia marcescens. Antibiotics. 2024; 13(4):323. [DOI:10.3390/antibiotics13040323] [PMID] [PMCID]
39. Khalil MI, Al-Tobje MA, Faisal RM. Molecular detection of virulence genes of Serratia marcescens isolates from diverse clinical sources. UNEC J Eng Appl Sci. 2024;4(2):99-105. [DOI:10.61640/ujeas.2024.1210]
40. Rizi KS, Hasanzade S, Soleimanpour S, Youssefi M, Jamehdar SA, Ghazvini K, et al. Phenotypic and molecular characterization of antimicrobial resistance in clinical species of Enterobacter, Serratia, and Hafnia in Northeast Iran. Gene Rep. 2021;25:101352. [DOI:10.1016/j.genrep.2021.101352]
41. Caliskan-Aydogan O, Alocilja EC. A review of carbapenem resistance in Enterobacterales and its detection techniques. Microorganisms. 2023; 11(6):1491. [PMID] [PMCID] [DOI:10.3390/microorganisms11061491]
42. Rizal NS, Neoh HM, Ramli R, Hanafiah A, Samat MN, Tan TL, et al. Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: perspectives from a middle-income country. Diagnostics. 2020; 10(10):816. [PMID] [PMCID] [DOI:10.3390/diagnostics10100816]
43. Uelze L, Grützke J, Borowiak M, Hammerl JA, Juraschek K, Deneke C, et al. Typing methods based on whole genome sequencing data. One Health Outlook. 2020;2(1):3. [PMID] [PMCID] [DOI:10.1186/s42522-020-0010-1]
44. Gambino AS, Déraspe M, Álvarez VE, Quiroga MP, Corbeil J, Roy PH, et al. Serratia marcescens SCH909 as reservoir and source of genetic elements related to wide dissemination of antimicrobial resistance mechanisms. FEMS Microbiol Lett. 2021;368(14):fnab086. [DOI:10.1093/femsle/fnab086] [PMID]
45. Kim UJ, Choi SM, Kim MJ, Kim S, Shin SU, Oh SR, et al. Hospital water environment and antibiotic use: key factors in a nosocomial outbreak of carbapenemase-producing Serratia marcescens.

J Hosp Infect. 2024;151:69-78. [DOI:10.1016/j.jhin.2024.04.021] [PMID]

46. Ji B, Ye W. Prevention and control of hospital-acquired infections with multidrug-resistant organism: A review. Medicine. 2024;103(4): e37018. [DOI:10.1097/MD.0000000000037018] [PMID] [PMCID]

47. Cosimato I, Santella B, Rufolo S, Sabatini P, Galdiero M, Capunzo M, et al. Current epidemiological status and antibiotic resistance profile of Serratia marcescens. Antibiotics. 2024; 13(4):323. [DOI:10.3390/antibiotics13040323] [PMID] [PMCID]

48. Liang Z, Shen J, Liu J, Sun X, Yang Y, Lv Y, et al. Prevalence and characterization of Serratia marcescens isolated from clinical bovine mastitis cases in Ningxia Hui autonomous region of China. Infection and Drug Resistance. 2023;16:2727-35. [DOI:10.2147/IDR.S408632] [PMID] [PMCID]

49. Khayyat AN, Hegazy WA, Shaldam MA, Mosbah R, Almalki AJ, Ibrahim TS, Khayat MT, Khafagy ES, Soliman WE, Abbas HA. Xylitol inhibits growth and blocks virulence in Serratia marcescens. Microorganisms. 2021;9(5):1083. [PMCID] [DOI:10.3390/microorganisms9051083] [PMID]