Shahid Bahonar University of Kerman
Agricultural Biotechnology Journal
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

Genotyping of clinical *Serratia marcescens* isolates using molecular techniques

Kazar Aintizar

Department of Biology, College of Education, University of Al-Qadisiyah, Iraq. E-mail address: aintizar.kazar@qu.edu.iq

Hussein Azhar

*Corresponding author: Department of Biology, College of Education, University of Al-Qadisiyah, Iraq. E-mail address: azhar.almousawi@qu.edu.iq

Abstract

Objective

Serratia marcescens is a Gram-negative, facultatively anaerobic bacillus belonging to the Enterobacteriaceae family. It is an opportunistic pathogen that has gained increasing attention due to its involvement in a wide range of nosocomial and community-acquired infections, containing urinary tract infections, respiratory tract infections, bloodstream infections, and wound infections. The bacterium is also known for its capability to survive in diverse environmental situations and its intrinsic resistance to different antibiotics, making its infections particularly challenging to treat in clinical settings. The 16S ribosomal RNA (16S rRNA) gene, which is highly conserved among bacteria but also contains hypervariable regions, is widely applied for phylogenetic investigations and bacterial identification. Genotyping applying PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the 16S rRNA gene permits differentiation of bacterial strains based on the presence of specific restriction sites. This technique prepares insights into genetic diversity and evolutionary relationships among isolates. The aim of this investigation was to genotype clinical isolates of *S. marcescens* achieved from various healthcare sources in Al-Diwaniyah Governorate, Iraq applying techniques like PCR, sequencing, and PCR-RFLP with the restriction enzymes *AluI* and *MspI*, and investigate the genetic diversity of local isolates.

Materials and methods

A total of 200 clinical samples were gathered from patients attending Diwaniyah Teaching Hospital as well as from different private medical clinics across Al-Diwaniyah Governorate, Iraq. Samples were inoculated onto standard selective and differential media. Genomic DNA was

extracted from purified *S. marcescens* isolates applying a commercial bacterial DNA extraction kit. Molecular identification was carried out by amplifying the 16S rRNA gene applying universal bacterial primers. PCR products of confirmed *S. marcescens* isolates were purified and sent to a commercial sequencing facility in South Korea. Phylogenetic trees were constructed applying the neighbor-joining method. To evaluate genetic variability among *S. marcescens* isolates, PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the 16S rRNA gene was carried out. Distinct RFLP patterns were analyzed visually, and the number of different genotypes was determined based on banding profiles for each enzyme.

Results

A total of 20 isolates of Serratia marcescens were achieved from different sources as follows: 15 (75%) isolates from urinary tract infections, 3 (15%) isolates from burn and wound injuries, and 2 (10%) isolates from eye injuries.

Conclusions

The investigation showed that molecular techniques prepared accurate data on the genetic makeup of Serratia marcescens, improving diagnostic accuracy, paving the way for the development of future diagnostic tools that are more sensitive and reliable.

Keywords: 16srRNA, genotyping, PCR-RFLP, Serratia marcescens

Paper Type: Research Paper.

Citation: Aintizar, K., & Azhar, H. (2025). Genotyping of clinical *Serratia marcescens* isolates applying molecular techniques. *Agricultural Biotechnology Journal 17*(2), 263-284.

Agricultural Biotechnology Journal 17 (2), 263-284. DOI: 10.22103/jab.2025.24830.1665

Received: March 07, 2025. Received in revised form: May 06, 2025.

Accepted: May 07, 2025. Published online: June 30, 2025.

Publisher: Faculty of Agriculture and Technology Institute of Plant Production, Shahid Bahonar University of Kerman-Iranian Biotechnology Society.

Introduction

Serratia marcescens is a Gram-negative, motile, facultatively anaerobic, non-spore-forming, catalase-positive, and oxidase-negative bacterium. Most species generate a red pigment named

prodigiosin, creating colonies to appear red or pink on solid nutrient media (Kljakić et al., 2020). It is a causative agent of opportunistic infections like pneumonia, meningitis, wound infections, respiratory tract infections, urinary tract infections, bacteremia, and corneal infections. The bloodstream is considered the most common site of infection when the gastrointestinal and respiratory systems are compromised (Kamali et al., 2024). Furthermore, *S. marcescens* is the fifth most common reason of nosocomial infections worldwide and the ninth most frequent microorganism isolated from bloodstream infections acquired in intensive care units across Europe (Hanczvikkel et al., 2024). *S. marcescens* generates different virulence factors, containing phospholipases, lipases, nucleases, chitinases, and proteases. It also has the capacity to form biofilms on both living and non-living surfaces, which facilitates colonization and persistence on medical devices like prostheses and catheters. Biofilm formation contributes significantly to the bacterium's increased resistance to antibiotics (Barman et al., 2020; Abbas and Hegazy, 2020). Based on recent investigations, *S. marcescens* exhibits resistance to a wide range of antibiotics, containing penicillins, cephalosporins, tetracyclines, macrolides, nitrofurantoin, and colistin. Historically, antibiotics like fluoroquinolones, aminoglycosides, and third-generation cephalosporins have been commonly applied for treating *S. marcescens* infections. However, many recent isolates from clinical cases display multiple forms of resistance to these antibiotics (Prado et al., 2022). In recent years, advanced molecular methods and techniques have been employed to investigate and diagnose bacterial communities. These techniques centralize on extracting and analyzing bacterial genetic material, often without requiring culture. Among them, the polymerase chain reaction (PCR) stands out as one of the most prominent molecular methods applied in diagnostics and forms the basis for other molecular techniques (Matys et al., 2024). PCR is widely applied in both research and clinical diagnosis; it works by amplifying specific DNA regions, which are then sequenced to analyze the nitrogenous bases and identify the bacterial species present in a sample (Riley et al., 2018). Furthermore, genetic diversity is vital for the development of more advanced genes, the preservation of populations, the progress of evolutionary processes, and the capability to adapt to changing environmental situations (Javanmard et al., 2008; Mohammadabadi et al., 2021a). Likewise, identifying gene polymorphisms is essential for disease detection and treatment (Mohammadabadi, 2016; Saadatabadi et al., 2023). Furthermore, investigating populations and breeds applying molecular techniques is important and beneficial for their characterization (Mohammadifar and Mohammadabadi, 2017; Noori et al., 2017; Mohammadinejad et al., 2022). Conservation of genetic diversity requires effective implementation of conservation priorities and sustainable management strategies, which should be based on comprehensive knowledge of population structures, containing genetic diversity both within and between populations and breeds

(Mohammadifar and Mohammadabadi, 2018; Mohammadabadi et al., 2024a). Genetic diversity is a fundamental component in genetic improvement, population preservation, evolution, and adaptation to varying environmental situations (Sulimova et al., 2007; Mohammadabadi et al., 2024b). Additionally, identifying gene polymorphisms is essential for characterizing various populations (Mohammadabadi et al., 2010; Mohammadabadi et al., 2024c), in order to define individual genotypes and their associations with the immune system, as well as their resistance or susceptibility to diseases (Mohammadabadi et al., 2021b; Shokri et al., 2023). Thus, given the significant role these bacteria play in nosocomial infections and various clinical situations, this investigation was conducted to investigate the prevalence of *S. marcescens* and to examine certain genetic aspects by genotyping the bacterial isolates applying a range of molecular techniques.

Materials and methods

Sample collection: A total of 200 clinical samples were gathered from various sources as follows: 25 (12.5%) eye injury samples, 25 (12.5%) burn and wound samples, and 150 (75.0%) urinary tract infection (UTI) samples. These samples were achieved between 11 March 2024 and 20 August 2024 from Diwaniyah Teaching Hospital in the Diwaniyah Governorate (Iraq), as well as from different private medical clinics within the same governorate.

Diagnosis: Initial diagnoses were carried out applying a range of culture media, containing nutrient agar, blood agar, and MacConkey agar. Microscopic examination was conducted in parallel with biochemical tests, which included the catalase test, oxidase test, IMViC test series, urease test, and triple sugar iron (TSI) test (Pérez-Viso et al., 2021). Final confirmation of isolates was carried out applying a phytic apparatus (Vijayaraghavan et al., 2013).

Molecular diagnosis of isolates: Genomic DNA was extracted from 20 bacterial isolates applying the Geneaid Genomic DNA Extraction Kit (Indonesia), following the manufacturer's instructions. Molecular identification was carried out via polymerase chain reaction (PCR), targeting the 16S rRNA gene sequence. The specific primers applied were (Sciesielski et al., 2023):

Forward: 5′-AGAGAGTTTGATCMTGGCTCAG-3′, Reverse: 5′-TACGGYTACCTTGTTACGACTT-3′.

The PCR products for the 16S rRNA diagnostic gene were subsequently sent to South Korea for DNA sequencing. The resulting sequences were submitted to NCBI GenBank, and a phylogenetic tree was generated applying the Neighbor-Joining method to compare our 16S rRNA sequences with reference sequences retrieved from the database. The Jukes–Cantor (JC) substitution model was applied for distance calculation. To evaluate the reliability of the tree,

bootstrap analysis was carried out with 1000 replications, and the resulting groupings reflected taxa associations supported by the bootstrap values (Idris et al., 2020).

PCR-RFLP analysis: PCR–restriction fragment length polymorphism (PCR-RFLP) analysis was carried out to differentiate the genotypes of the 16S rRNA gene among the 20 bacterial isolates. The amplified 16S rRNA gene fragments were subjected to enzymatic digestion applying two restriction endonucleases: *AluI* and *MspI*. These enzymes were selected based on their known capability to generate polymorphic restriction patterns suitable for distinguishing closely related bacterial species. Each digestion reaction was carried out in a final volume of 40 µL, consisting of 10 µL of the purified PCR product, 5 µL of the respective restriction enzyme (as per manufacturer's instructions), and nuclease-free distilled water to complete the volume. The reaction mixtures were incubated under situations optimized for each enzyme to ensure complete digestion. The resulting DNA fragments were separated via electrophoresis on a 2% agarose gel containing 0.5 µg/mL ethidium bromide. A molecular size marker (DNA ladder) with fragments up to 2000 base pairs was included to permit for accurate estimation of fragment sizes. Distinct RFLP patterns were observed across the isolates, indicating genetic diversity within the 16S rRNA gene region. These patterns prepare a preliminary basis for classification and phylogenetic analysis of the isolates. The method follows the approach described by Rohit et al. (2016), with minor modifications for optimization. This molecular typing technique proved to be a reliable, cost-effective method for discriminating among bacterial genotypes, especially in cases where sequencing resources are limited. The observed polymorphisms reflect underlying genetic variation, potentially correlating with phenotypic or ecological differences among the isolates.

Sample collection: A total of 200 clinical samples were gathered from various sources between March 11, 2024, and August 20, 2024. The specimens were achieved from patients at Diwaniyah Teaching Hospital, located in the Diwaniyah Governorate of Iraq, as well as from different private medical clinics within the same region. The distribution of the samples was as follows: 25 samples (12.5%) were gathered from patients with eye injuries, 25 samples (12.5%) from burn and wound infections, and 150 samples (75.0%) from individuals diagnosed with urinary tract infections (UTIs). All samples were gathered under aseptic situations and transported to the laboratory under cold chain to preserve sample integrity. Ethical approval was achieved prior to sample collection, and patient consent was ensured in accordance with institutional and national guidelines. The diversity of sample sources prepared a broad representation of common clinical infections in the region, supporting comprehensive microbiological and molecular analysis.

Diagnosis: Initial identification of bacterial isolates was conducted applying a combination of conventional microbiological techniques. Clinical specimens were cultured on various

selective and differential media, containing nutrient agar, blood agar, and MacConkey agar, to promote the growth and preliminary differentiation of potential pathogens based on colony morphology, hemolytic activity, and lactose fermentation profiles. Following incubation, isolates were subjected to microscopic examination applying Gram staining to evaluate cellular morphology and Gram reaction. Further biochemical characterization was carried out applying a panel of standard diagnostic tests, which included the catalase test, oxidase test, and the IMViC series (Indole, Methyl Red, Voges–Proskauer, and Citrate utilization tests). Additional assays like the urease test and the Triple Sugar Iron (TSI) test were also employed to support species-level identification. These biochemical tests were carried out applying commercially available kits (Microxpress®; A Division of Tulip Diagnostics (P) Ltd., India), following the manufacturer's instructions.

Molecular diagnosis of isolates: Genomic DNA was extracted from 20 selected bacterial isolates applying the Geneaid Genomic DNA Extraction Kit, following the protocol described by Pazla et al. (2024). The molecular identification of these isolates was conducted through polymerase chain reaction (PCR), targeting the highly conserved 16S rRNA gene region. Amplification was carried out applying universal bacterial primers: forward primer 5′-AGAGAGTTTGATCMTGGCTCAG-3′ and reverse primer 5′-TACGGYTACCTTGTTACGACTT-3′, as reported by Sciesielski et al. (2023). PCR amplification situations were optimized to ensure specificity and yield of the target fragment. The amplified products were visualized on agarose gel electrophoresis to confirm successful amplification. Subsequently, the PCR products were purified and sent to Macrogen Inc. (Seoul, South Korea) for Sanger sequencing. The resulting sequences were submitted to the NCBI GenBank database, and accession numbers were achieved for each isolate. To infer the evolutionary relationships among the bacterial isolates, a phylogenetic tree was constructed applying the Neighbor-Joining method with the Jukes–Cantor (JC) substitution model, based on a Maximum Likelihood (ML) framework, as described by Idris et al. (2020). This molecular approach enabled precise taxonomic classification and prepared insights into the genetic relatedness among the isolates, supplementing the phenotypic and biochemical diagnostic data.

PCR-RFLP analysis: PCR–restriction fragment length polymorphism (PCR-RFLP) analysis was conducted to differentiate the genotypes of the 16S rRNA gene among the 20 bacterial isolates. Amplified 16S rRNA gene products were digested applying two restriction endonucleases: *AluI* and *MspI*. These enzymes were selected for their capability to recognize specific nucleotide sequences and generate distinct fragment patterns suitable for genotypic differentiation. Each digestion reaction was prepared in a total volume of 40 µL, consisting of 15

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

µL of the PCR amplification product, 5 µL of the respective restriction enzyme, and nuclease-free distilled water to bring the volume to 40 µL. The reactions were incubated under optimal situations recommended by the enzyme manufacturers to ensure complete digestion of the DNA. Following digestion, the samples were subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide at a final concentration of 0.5 µg/mL. A DNA ladder with fragment sizes up to 2000 base pairs was applied as a molecular size reference. The electrophoretic separation revealed distinct restriction patterns for the different isolates, indicating variability within the amplified 16S rRNA gene region. This PCR-RFLP approach proved to be a reliable method for evaluating genetic diversity among bacterial isolates. The protocol followed the methodology described by Rohit et al. (2016), with modifications to optimize digestion and visualization situations for the target gene.

Results and discussion

Isolation and identification of *Serratia marcescens:* A total of 20 clinical isolates of *Serratia marcescens* were successfully achieved from various infection sites. Among these, 15 isolates (75%) were derived from patients with urinary tract infections (UTIs), 3 isolates (15%) from burn and wound infections, and 2 isolates (10%) from eye infections (Figure 1). These findings highlight the predominance of *S. marcescens* in urinary tract infections, consistent with previous reports indicating its frequent involvement in nosocomial UTIs due to its capability to colonize catheterized urinary tracts and persist in hospital environments. Molecular identification applying polymerase chain reaction (PCR) targeting the 16S rRNA gene confirmed the identity of all 20 isolates. Electrophoretic analysis of PCR products revealed a single distinct band corresponding to a molecular weight of approximately 1500 base pairs in all samples (Figure 2). This size is consistent with the expected amplicon length for the 16S rRNA gene in *S. marcescens*, thereby confirming successful amplification and supporting the specificity of the primers applied. The consistent amplification across all isolates suggests high genetic conservation in the 16S rRNA region among the studied strains, reinforcing its reliability as a diagnostic marker for species-level identification. Furthermore, the clear and specific PCR bands without non-specific amplification products underscore the effectiveness of the DNA extraction and amplification protocols employed. The capability to isolate *Serratia marcescens* from diverse sources reflects its broad pathogenic potential and highlights the bacterium's high pathogenicity and varied virulence mechanisms. As an opportunistic pathogen, *S. marcescens* is known to create a wide range of infections in humans, particularly in immunocompromised individuals. It is capable of spreading epidemiologically, leading to infections in hospitalized patients. These include cystitis, arthritis, eye and respiratory tract infections, urinary tract infections, sepsis, and meningitis. The

bacterium is often associated with infections in individuals undergoing treatment with broad-spectrum antibiotics, particularly those involving invasive procedures like the use of intravenous and urinary catheters (Cristina et al., 2019; Sader et al., 2014).

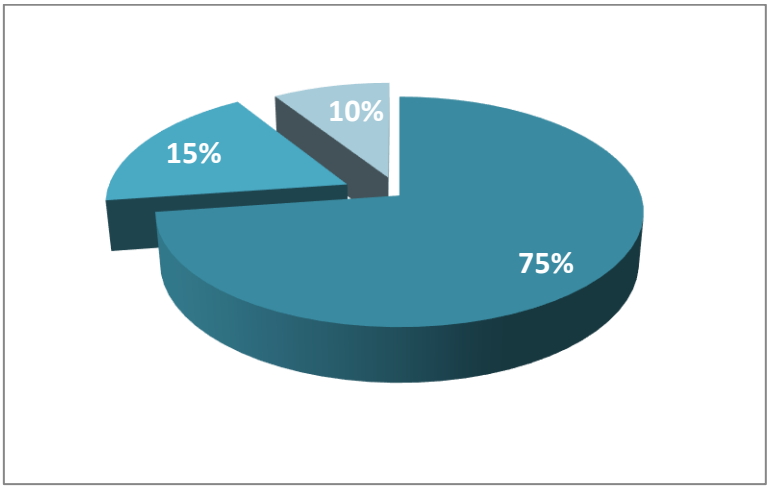

Figure 1. Distribution of *Serratia marcescens* isolates achieved from clinical samples: urinary tract infections (75%), burn and wound injuries (15%), and eye infections (10%).

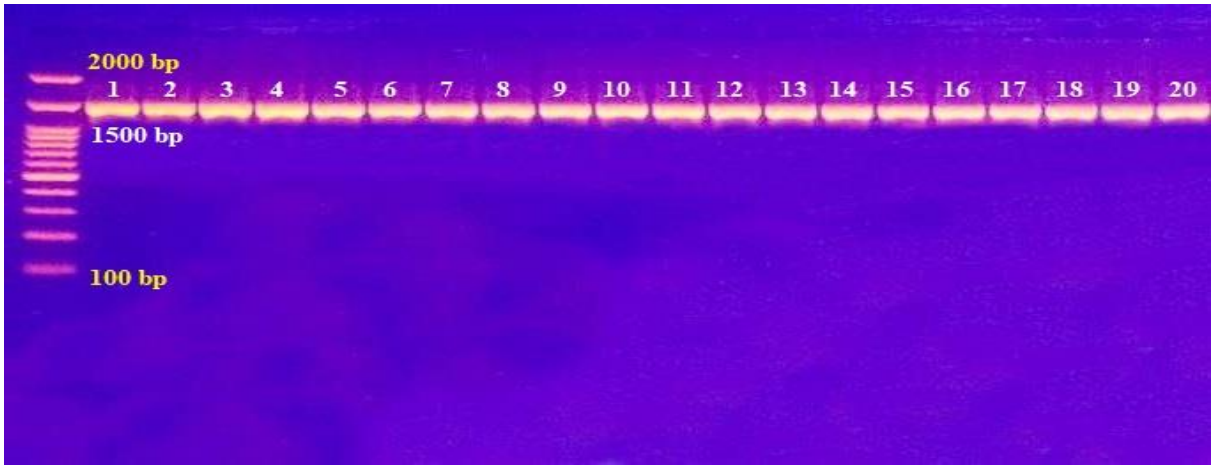

Figure 2. Agarose gel electrophoresis of PCR products amplifying the 16S rRNA gene of *Serratia marcescens* isolates. A single band of ~1500 base pairs is observed for all isolates (lanes 1–20). M: molecular weight DNA ladder (100–2000 bp). Electrophoresis was carried out at 80 V.

The results of this investigation are in agreement with the findings of Al-Musawi (2018), who reported an incidence of *S. marcescens* in diarrheal samples at 73.5%. Our investigation's results are also comparable to those of Salem and Al-Bayati (2023) in Wasit City, where *S. marcescens* was isolated from 69.8% of samples, with 18 isolates achieved from 207 bacterial

isolates from various clinical sources. These results slightly exceed those reported by Anfal et al. (2011), who isolated *S. marcescens* in 57% of UTI cases. Conversely, the prevalence observed in this investigation differs from that reported by Awayid and Aamzah (2023), who isolated *S. marcescens* in only 18.18% of their patient samples. Similarly, Muslim et al. (2017) reported a lower isolation rate of 5.8% from wound infections, while Daham (2021) achieved 41 isolates, although the specific prevalence was not stated.

The variation in results across these investigations can be attributed to different factors, containing differences in the sample size, patient population, healthcare practices, and geographical regions. Additionally, factors like local environmental situations, patient age groups, social and cultural factors, and levels of health awareness may contribute to the differing isolation rates observed. Furthermore, the genetic diversity and virulence of *S. marcescens* strains, depending on their source, may influence their prevalence in different clinical settings.

The molecular diagnostic results of this investigation were consistent with those achieved from traditional diagnostic methods, like FITC, culture, microscopy, and biochemical tests. PCR amplification of the 16S rRNA gene yielded a single, clear band of approximately 1500 base pairs for each isolate (Figure 2). The appearance of this band can be attributed to the successful binding of the primers to conserved regions within the *S. marcescens* 16S rRNA gene. The specificity of the primers applied is further evidenced by the uniformity and clarity of the PCR bands across all isolates, underscoring the high specificity of the 16S rRNA diagnostic assay.

Molecular diagnostics, particularly applying PCR to amplify the 16S rRNA gene, have been employed with great success in the identification of *S. marcescens* isolates in previous investigations (Kashash, 2021). These investigations demonstrated the high diagnostic accuracy of PCR compared to conventional methods. The advantages of PCR include its rapid processing time, high sensitivity, and specificity, which make it a powerful tool in clinical microbiology. PCR-based diagnostics reduce the need for specialized culture media, permit for the identification of viable but non-culturable organisms, and prepare the opportunity for long-term storage of genetic material for future analysis (Riegman et al., 2019).

To further confirm the genetic identity of the isolates, the PCR products of the 16S rRNA gene were sent to Korea for sequencing. The sequences were then analyzed and matched applying the NCBI BLAST program. Each of the 20 isolates was registered in the National Center for Biotechnology Information (NCBI) database, and unique accession numbers were achieved for each strain. Phylogenetic analysis of these isolates was carried out applying the MEGA Molecular Evolutionary Genetics Analysis (MEGA) software, and a phylogenetic tree was constructed (Figure 3). The tree illustrates the genetic relatedness of the investigation isolates to globally

sourced *S. marcescens* strains, highlighting similarities and potential variations in the strains' genetic makeup.

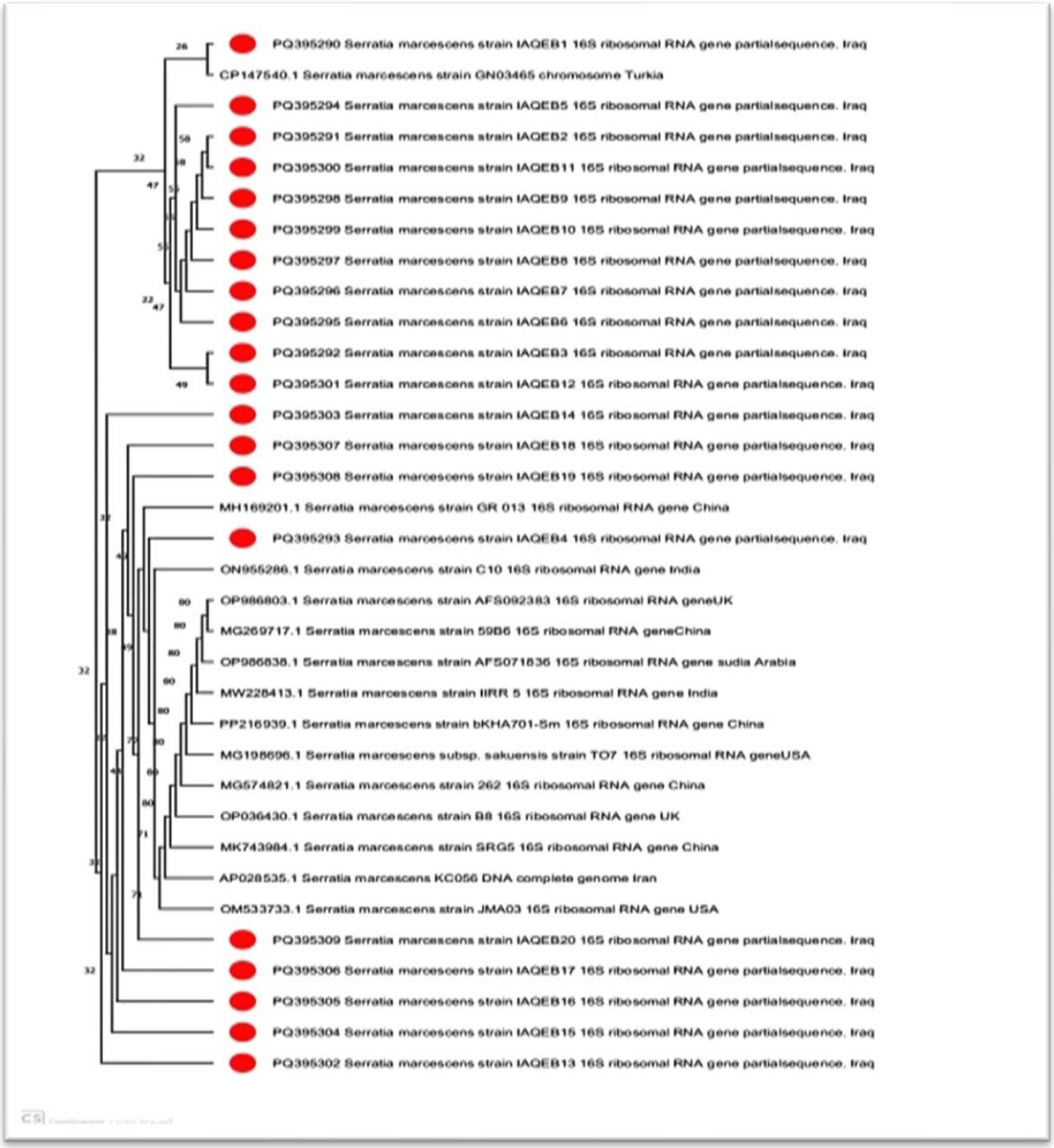

Figure 3. Phylogenetic tree analysis of the 16S rRNA gene sequences of the *Serratia marcescens* isolates studied, compared with global reference isolates. The tree illustrates the genetic relationships and similarity percentages between the local isolates and globally available *S. marcescens* strains, providing insight into their genetic diversity and evolutionary relationships.

We observed that the studied local *Serratia marcescens* isolates, indicated by red circles in the phylogenetic tree, tend to cluster closely together, suggesting a high degree of genetic similarity among them. For instance, isolate PQ395290 exhibits a notable genetic similarity to the global isolate CP147540.1 from Turkey. Furthermore, the local isolates can be grouped into two main clusters: one cluster shows strong affinity with isolates from China and India, like OP986833.1 and MG547821.1, while the second cluster is more closely related to isolates from the United States and the United Kingdom, containing OM533733.1 and OP036430.1. This pattern of genetic similarity may reflect a global spread of specific *S. marcescens* strains. The clustering of local isolates with those from Turkey and India could be explained by factors like the importation of medical devices, the movement of patients traveling for treatment, and the international exchange of medical supplies. *S. marcescens*, an opportunistic pathogen, can exploit primary infections, especially those resulting from renal failure, which may also be transmitted through contaminated medical supplies. Notably, *S. marcescens* is responsible for approximately 2% of hospital-acquired infections, like urinary tract infections (Posluszny et al., 2011).

Genotyping of *Serratia marcescens* isolates applying 16S rRNA gene polymorphism: The genotyping of the *S. marcescens* isolates was carried out applying the PCR-restriction fragment length polymorphism (PCR-RFLP) technique to detect variations in the 16S rRNA gene. The results of this analysis are presented in Figures 4 and 5.

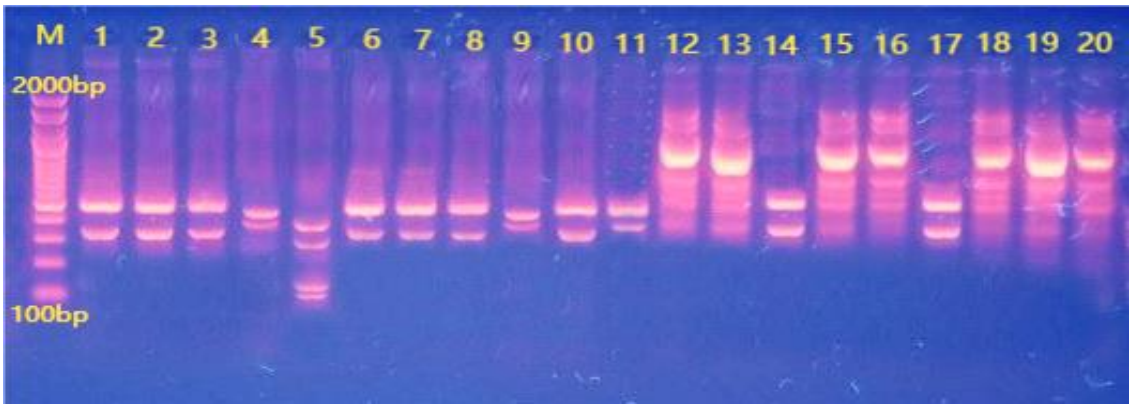

Figure 4. Electrophoresis of PCR-RFLP products from the 16S rRNA gene of 20 *S. marcescens* isolates, applying the AluI restriction enzyme. The gel was run at a concentration of 2% agarose, with a voltage of 100 mV and a potential difference of 80 A for 1 hour. The isolates are numbered (1-20), and the "M" lane represents the DNA ladder.

The characterization of *Serratia marcescens* isolates based on the RFLP-PCR technique, applying the AluI restriction enzyme, revealed the formation of six distinct patterns, as shown in

Table 1. These patterns are coded as S1, S2, S3, S4, S5, and S6. The total fragment sizes ranged from 100 to 600 base pairs. Among these, the S1 and S2 patterns were the most prevalent, each appearing in seven isolates (35% frequency). Specifically, the S1 pattern was found in isolates 1, 2, 3, 6, 7, 8, and 10, while the S2 pattern was observed in isolates 12, 13, 15, 16, 19, and 20. The S3 and S4 patterns were each observed in two isolates, corresponding to a frequency of 10%. The S3 pattern was detected in isolates 4 and 9, while the S4 pattern appeared in isolates 14 and 17. Finally, the S5 and S6 patterns were the least common, each appearing in only one isolate (5% frequency), with S5 found in isolate 5 and S6 in isolate 11.

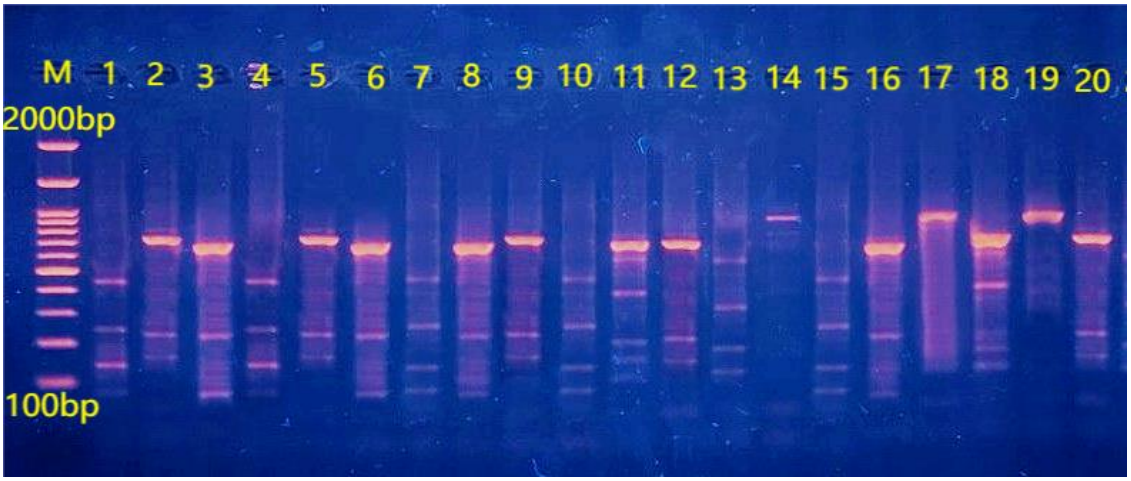

Figure 5. Electrophoresis of PCR-RFLP products from the 16S rRNA gene of 20 *S. marcescens* isolates, applying the MspI restriction enzyme. The gel was prepared with 2% agarose, and the electrophoresis was carried out at a voltage of 100 mV and a potential difference of 80 A for 1 hour. The isolates are numbered (1-20), and the "M" lane represents the DNA ladder.

Table 1. RFLP patterns of the 16S rRNA gene of the studied *Serratia marcescens* isolates, applying the AluI restriction enzyme.

Genotype	Number of isolates	Average size of the resulting genotypes	Frequency ratio
S1	7(1,2,3,6,7,8,10)	300-500 bp	35 %
S2	7(12,13,15,16,18,19,20)	400 1500 bp	35 %
S3	2(4,9)	400-450 bp	10 %
S4	2(14,17)	350-600 bp	10 %

S5	1(5)	100-400 bp	5 %
S6	1(11)	400-500 bp	5 %

For the characterization of *Serratia marcescens* isolates applying the MspI restriction enzyme, a distinct set of genotypes was observed, as summarized in Table 2. Five different genotypes were identified, coded as M1, M2, M3, M4, and M5. The fragment sizes ranged from 100 to 750 base pairs. The M1 and M2 patterns were the most common, each appearing in five isolates, corresponding to a frequency of 25%. The M1 pattern was present in isolates 2, 5, 9, 12, and 20, while the M2 pattern was observed in isolates 1, 4, 7, 10, and 15. The M3 pattern appeared in four isolates with a frequency of 20% (isolates 3, 6, 8, and 16). The M4 pattern was found in isolates 11 and 18, with a frequency of 10%, while the M5 pattern was observed in only one isolate (isolate 12), corresponding to a frequency of 5%. Isolates 14, 17, and 19 did not exhibit any bands when applying the MspI restriction enzyme. This lack of detection may be due to the absence or loss of restriction enzyme binding sites within the diagnostic gene of these isolates. Such a loss can occur in variable regions of bacterial genes, particularly in regions of tandem repeats, which are prone to changes that can affect enzyme recognition sites.

Table 2. RFLP patterns of the 16S rRNA gene of the studied *Serratia marcescens* isolates, applying the MspI restriction enzyme

Genotype	Number of isolates	Average size of the resulting genotypes	Frequency ratio
M1	5(2,5,9,12,20)	150-750 bp	25%
M2	5(1,4,7,10,15)	100-450 bp	25%
M3	4(3,6,8,16)	100-700 bp	20%
M4	2(11,18)	150-700 bp	10%
M5	1(13)	150-600 bp	5%

PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) is a molecular biology technique that integrates the polymerase chain reaction (PCR) with digestion of the PCR product by restriction enzymes to analyze and identify genetic differences between bacterial isolates. This method is particularly valued for its simplicity, cost-effectiveness, and capability to deliver rapid results across a range of applications. The technique involves four main steps: isolation of the genetic material, amplification of the target DNA, digestion of the PCR

product applying restriction enzymes, and analysis of the resulting fragments through electrophoresis. By combining PCR amplification with restriction fragment length polymorphism (RFLP) analysis, PCR-RFLP enables the characterization of genetic variations, offering a straightforward and accurate alternative to other techniques, like sequencing or hybridization (Tarach, 2021). The RFLP technique has been employed in various investigations to investigate genetic relationships and diagnose bacterial infections. For instance, in a investigation by Mehdi et al. (2019) conducted in Iran, RFLP was applied to determine the genetic relationships among 20 *Campylobacter jejuni* isolates from children with diarrhea. The investigation concluded that genotyping the flaA gene applying the DdeI enzyme was a rapid, cost-effective, and reliable method for the epidemiological investigation of clinical *C. jejuni* isolates, particularly in areas with limited resources and for large-scale population surveillance. Similarly, in a investigation by Rohit et al. (2016), RFLP of the 16S rRNA gene was utilized to identify the causative organisms of neonatal septicemia, with results compared to blood culture findings. Blood samples from 97 newborns suspected of septicemia were tested, and the investigation revealed the superiority of the RFLP technique. RFLP successfully diagnosed 55 cases, whereas blood cultures were positive in only 34 cases. The investigation also found that *Staphylococcus aureus* was the most common organism creating sepsis based on both culture and molecular diagnostics. *Klebsiella spp.* was isolated from four samples via culture but was detected in five cases applying PCR-RFLP, and *Acinetobacter spp.* was isolated from one case by culture but was identified in eight cases applying PCR-RFLP. These findings led the investigation to recommend the adoption of PCR-RFLP technology for rapid results and for its potential in early diagnosis of blood poisoning in newborns, even when blood cultures are negative.

Conclusions: This investigation yielded significant insights into the genotypic diversity of *Serratia marcescens*, revealing clear genetic variation among the clinical isolates analyzed. Through the application of advanced molecular techniques, containing polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP), six distinct 16S rRNA genotypes were identified applying the AluI restriction enzyme and five genotypes applying the MspI enzyme. These findings underscore the value of molecular diagnostics in providing high-resolution differentiation between bacterial strains, especially for opportunistic pathogens like *S. marcescens*. The demonstrated genetic diversity among the isolates highlights the importance of adopting molecular approaches in routine microbial surveillance and diagnostics. Furthermore, the registration of the isolates in the Global GenBank database adds substantial scientific merit, creating a genetic reference point for future comparative and epidemiological investigations. This advancement not only deepens our understanding of *S. marcescens* biology but also supports

efforts to identify antibiotic-resistant strains and track those implicated in hospital-acquired infections. Overall, the investigation contributes to the growing body of knowledge necessary for the development of targeted diagnostic tools and more effective infection control strategies.

Author Contributions

Hussein Azhar and Kazar Aintizar designed the investigation; Hussein Azhar prepared experimental samples; Hussein Azhar and Kazar Aintizar analysed the data; Hussein Azhar wrote the paper. All authors read and approved the final manuscript.

Data Availability Statement

The data can be prepared by the corresponding author on reasonable request.

Acknowledgements

Authors would like to acknowledge all the support prepared by the University of Al-Qadisiyah.

Ethical Considerations

Approval to conduct this investigation was achieved from the Iraqi Ministry of the Health Research Department, University of Al-Qadisiyah, Department of Biology ethical review board, and Research Ethics Committees of hospitals.

Funding

This work has been funded by the Natural Science Foundation of University of Al-Qadisiyah (Grant No. 2024AQ050198),

Conflict of Interest

The authors declare that there are no conflicts of interest

References

Anfal, M. K., Suhaila, S., & Mohammed, A. F. (2011). Isolation of Multi Antibiotic Resistance *Serratia marcescens* and the Detection of AmpC & GESL Genes by Polymerase Chain Reaction Technique. *Al-Mustansiriyah Journal of Science* 22(6), 329-346. https://doi.org/10.23851/mjs.v22i6

Barman, S., Bhattacharya, S. S., & Mandal, N. C. (2020). Serratia. In N. Amaresan, M. S. Kumar, K. Annapurna, K. Kumar, & A. Sankaranarayanan (Eds.), *Beneficial microbes in agro-*

ecology (pp. 27–36). Academic Press. https://doi.org/10.1016/B978-0-12-823414-3.00003-4

Cristina, M. L., Sartini, M., & Spagnolo, A. M. (2019). *Serratia marcescens* infections in neonatal intensive care units (NICUs). *International Journal of Environmental Research and Public Health, 16*(4), 610. https://doi.org/10.3390/ijerph16040610

Daham, R. I. (2021). Daham, R. I. (2021). *Immunological and biological effects of Serratia marcescens extracellular protease isolated from UTI infections* (Master's thesis, Mustansiriyah University).

Ghorbanalizadgan, M., Bakhshi, B., Shams, S., & Najar-Peerayeh, S. (2019). Ghorbanalizadgan, M., Bakhshi, B., Shams, S., & Najar-Peerayeh, S. (2019). Pulsed-field gel electrophoresis fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from clinical specimens, Iran. *International Microbiology, 22*(3), 391–398. https://doi.org/10.1007/s10123-019-00073-2

Hamzah, A. S., & Awayid, H. S. (2023). Hamzah, A. S., & Awayid, H. S. (2023). Analysis of virulence genes sequencing of *Serratia marcescens* in Iraqi hospitals. *Cellular and Molecular Biology, 69*(11), 162–166. https://doi.org/10.14715/cmb/2023.69.11.27

Hanczvikkel, A., Tóth, Á., Németh, I. A. K., Bazsó, O., Závorszky, L., Buzgó, L., ... & Hajdu, Á. (2024). Hanczvikkel, A., Tóth, Á., Németh, I. A. K., Bazsó, O., Závorszky, L., Buzgó, L., ... & Hajdu, Á. (2024). Nosocomial outbreak caused by disinfectant-resistant *Serratia marcescens* in an adult intensive care unit, Hungary, February to March 2022. *Eurosurveillance, 29*(26), 2300492. https://doi.org/10.2807/1560-7917.ES.2024.29.26.2300492

Idris, A. B., Hassan, H. G., Ali, M. A. S., Eltaher, S. M., Idris, L. B., Altayb, H. N., Abass, A. M., Ibrahim, M. M. A., Ibrahim, E. M., & Hassan, M. A. (2020). Molecular phylogenetic analysis of 16S rRNA sequences identified two lineages of *Helicobacter pylori* strains detected from different regions in Sudan suggestive of differential evolution. *International Journal of Microbiology, 2020*, 8825718. https://doi.org/10.1155/2020/8825718

Javanmard, A., Mohammadabadi, M. R., Zarrigabayi, G. E., & Gharahedaghi, A. A. (2008). Polymorphism within the intron region of the bovine leptin gene in Iranian Sarabi cattle (*Bos taurus*). *Russian Journal of Genetics, 44*(4), 495-497. https://doi.org/10.1134/S1022795408040169

Kamali, A., Ferguson, D., Dowless, H., Ortiz, N., Mukhopadhyay, R., Schember, C., ... & Kimura, A. (2024). Kamali, A., Ferguson, D., Dowless, H., Ortiz, N., Mukhopadhyay, R., Schember, C., ... & Kimura, A. (2024). Outbreak of invasive *Serratia marcescens* among

persons incarcerated in a state prison, California, USA, March 2020–December 2022. *Emerging Infectious Diseases, 30*(Suppl 1), S41. https://doi.org/10.3201/eid3001.231234

Kashash, R. R. (2021). Kashash, R. R. (2021). *Isolation and molecular identification of Serratia marcescens from different clinical sources in humans and birds* (Master's thesis, University of Baghdad).

Kljakić, D., Milosavljević, M. Z., Jovanović, M., Popović, V. Č., & Raičević, S. (2020). Kljakić, D., Milosavljević, M. Z., Jovanović, M., Popović, V. Č., & Raičević, S. (2020). *Serratia marcescens* as a cause of unfavorable outcome in the twin pregnancy. *Open Medicine, 16*(1), 81–86. https://doi.org/10.1515/med-2021-0001

Matys, J., Kensy, J., Gedrange, T., Zawiślak, I., Grzech-Leśniak, K., & Dobrzyński, M. (2024). Matys, J., Kensy, J., Gedrange, T., Zawiślak, I., Grzech-Leśniak, K., & Dobrzyński, M. (2024). A molecular approach for detecting bacteria and fungi in healthcare environment aerosols: A systematic review. *International Journal of Molecular Sciences, 25*(8), 4154. https://doi.org/10.3390/ijms25084154

Mohammadabadi, M. (2016). Inter-simple sequence repeat loci associations with predicted breeding values of body weight in kermani sheep. *Genetics in the Third Millennium, 14*(4), 4386-4393. https://sciexplore.ir/Documents/Details/472-591-539-924

Mohammadabadi, M. R., Torabi, A., Tahmourespoor, M., & Baghizadeh, A. (2010). Analysis of bovine growth hormone gene polymorphism of local and Holstein cattle breeds in Kerman province of Iran using polymerase chain reaction restriction fragment length. *African Journal of Biotechnology, 9*(41), 6848-6852. https://www.ajol.info/index.php/ajb/article/view/130242

Mohammadabadi, M., Akhtarpoor, A., Khezri, A., Babenko, O., Stavetska, R. V., Tytarenko, I., Ievstafiieva, Y., Buchkovska, V., Slynko, V. and Afanasenko, V. (2024a). The role and diverse applications of machine learning in genetics, breeding, and biotechnology of livestock and poultry. *Agricultural Biotechnology Journal, 16*(4), 413-442. https://doi.org/10.22103/jab.2025.24662.1644

Mohammadabadi, M., Babenko Ivanivna, O., Borshch, O., Kalashnyk, O., Ievstafiieva, Y. and Buchkovska, V. (2024c). Measuring the relative expression pattern of the UCP2 gene in different tissues of the Raini Cashmere goat. *Agricultural Biotechnology Journal, 16*(3), 317-332. https://doi.org/10.22103/jab.2024.24337.1627

Mohammadabadi, M., Kheyrodin, H., Afanasenko, V., Babenko Ivanivna, O., Klopenko, N., Kalashnyk, O., Ievstafiieva, Y., & Buchkovska, V. (2024b). The role of artificial intelligence in genomics. *Agricultural Biotechnology Journal, 16*(2), 195-279. https://doi.org/10.22103/jab.2024.23558.1575

Mohammadabadi, M., Meymandi, M.G., Montazeri, M., Afanasenko, V., & Kalashnyk, O. (2021a). Molecular characterization of Iranian dromedaries using microsatellite markers. *Acta Agronomica, 69*(4), 321-330. *https://api.semanticscholar.org/CorpusID:249364533*

Mohammadabadi, M., Oleshko, V., Oleshko, O., Heiko, L., Starostenko, I., Kunovskii, J., Bazaeva, A., & Roudbari, Z. (2021b). Using inter simple sequence repeat multi-loci markers for studying genetic diversity in guppy fish. *Turkish Journal of Fisheries and Aquatic Sciences, 21,* 603-613. http://doi.org/10.4194/1303-2712-v21_12_03

Mohammadifar, A., & Mohammadabadi, M. (2018). Melanocortin-3 receptor (MC3R) gene association with growth and egg production traits in fars indigenous chicken. *Malaysian Applied Biology, 47*(3), 85-90.

Mohammadifar, A., & Mohammadabadi, M. R. (2017). The effect of uncoupling protein polymorphisms on growth, breeding value of growth, and reproductive traits in the Fars indigenous chicken. *Iranian Journal of Applied Animal Science, 7*(4), 679-685. https://journals.iau.ir/article_535799.html

Mohammadinejad, F., Mohammadabadi, M., Roudbari, Z., & Sadkowski, T. (2022). Identification of Key Genes and Biological Pathways Associated with Skeletal Muscle Maturation and Hypertrophy in *Bos taurus*, *Ovis aries,* and *Sus scrofa*. *Animals*, *12*(24), 3471. https://doi.org/10.3390/ani12243471

Noori, A. N., Behzadi, M. R. B., & Mohammadabadi, M. R. (2017). Expression pattern of *Rheb* gene in Jabal Barez Red goat. *Indian Journal of Animal Science, 87*(11), 1375-1378. https://doi.org/10.56093/ijans.v87i11.75890

Pazla R, Yanti G, Jamarun N, Zain M, Triani HD, Putri EM, Srifani A. Identification of phytase producing bacteria from acidifying *Tithonia diversifolia*: Potential for ruminant feed development. Saudi J Biol Sci. 2024 Jul;31(7):104006. https://doi.org/10.1016/j.sjbs.2024.104006.

Pazla R, Yanti G, Jamarun N, Zain M, Triani HD, Putri EM, Srifani A. Identification of phytase producing bacteria from acidifying *Tithonia diversifolia*: Potential for ruminant feed development. Saudi J Biol Sci. 2024 Jul;31(7):104006. https://doi.org/10.1016/j.sjbs.2024.104006.

Pérez-Viso, B., Aracil-Gisbert, S., Coque, T. M., Del Campo, R., Ruiz-Garbajosa, P., & Cantón, R. (2021). Evaluation of CHROMagar™-Serratia agar, a new chromogenic medium for the detection and isolation of *Serratia marcescens*. *European Journal of Clinical Microbiology & Infectious Diseases, 40*(12), 2593–2596. https://doi.org/10.1007/s10096-021-04319-9

Posluszny, J. A., Jr., Conrad, P., Halerz, M., Shankar, R., & Gamelli, R. L. (2011). Surgical burn wound infections and their clinical implications. *Journal of Burn Care & Research, 32*(3), 324–333. https://doi.org/10.1097/BCR.0b013e31820aaf4f

Prado, L. C. D. S., Giacchetto Felice, A., Rodrigues, T. C. V., Tiwari, S., Andrade, B. S., Kato, R. B., ... & Soares, S. D. C. (2022). New putative therapeutic targets against *Serratia marcescens* using reverse vaccinology and subtractive genomics. *Journal of Biomolecular Structure and Dynamics, 40*(20), 10106–10121. https://doi.org/10.1080/07391102.2021.1931022

Riegman, P. H. J., Becker, K. F., Zatloukal, K., Pazzagli, M., Schröder, U., & Oelmuller, U. (2019). How standardization of the pre-analytical phase of both research and diagnostic biomaterials can increase reproducibility of biomedical research and diagnostics. *New Biotechnology, 53*, 35–40. https://doi.org/10.1016/j.nbt.2018.03.002

Riley, L. W. (2018). Laboratory methods in molecular epidemiology: Bacterial infections. *Microbiology Spectrum, 6*(6), 10–1128. https://doi.org/10.1128/microbiolspec.ARBA-0022-2017

Rohit, A., Maiti, B., Shenoy, S., & Karunasagar, I. (2016). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for rapid diagnosis of neonatal sepsis. *Indian Journal of Medical Research, 143*(1), 72–78. https://doi.org/10.4103/0971-5916.178618

Saadatabadi, L. M., Mohammadabadi, M., Nanaei, H. A., Ghanatsaman, Z. A., Stavetska, R. V., Kalashnyk, O., Kochuk-Yashchenko, O. A., & Kucher, D. M. (2023). Unraveling candidate genes related to heat tolerance and immune response traits in some native sheep using whole genome sequencing data. *Small Ruminant Research, 225*, 107018. https://doi.org/10.1016/j.smallrumres.2023.107018

Sader, H. S., Farrell, D. J., Flamm, R. K., & Jones, R. N. (2014). Antimicrobial susceptibility of Gram-negative organisms isolated from patients hospitalized in intensive care units in United States and European hospitals (2009–2011). *Diagnostic Microbiology and Infectious Disease, 78*(4), 443–448. https://doi.org/10.1016/j.diagmicrobio.2013.11.021

Sciesielski, L. K., Osang, L. K., Dinse, N., Weber, A., Bührer, C., Kola, A., & Dame, C. (2023). Validation of a new PCR-based screening method for prevention of *Serratia marcescens* outbreaks in the neonatal intensive care unit. *Neonatology, 120*(2), 176–184. https://doi.org/10.1159/000529230

Shokri, S., Khezri, A., Mohammadabadi, M., & Kheyrodin, H. (2023). The expression of *MYH7* gene in femur, humeral muscle, and back muscle tissues of fattening lambs of the Kermani breed. *Agricultural Biotechnology Journal, 15*(2), 217-236. https://doi.org/10.22103/jab.2023.21524.1486

Sulimova, G.E., Azari, M.A., Rostamzadeh, J., Mohammadabadi, M. R., & Lazebny, O. E. (2007). κ-casein gene (*CSN3*) allelic polymorphism in Russian cattle breeds and its information value as a genetic marker. *Russian Journal of Genetics*, 43, 73-79. https://doi.org/10.1134/S1022795407010115

Tarach, P. (2021). Application of polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) in the analysis of single nucleotide polymorphisms (SNPs). *Acta Universitatis Lodziensis. Folia Biologica et Oecologica, 17*, 48–53. https://doi.org/10.18778/1730-536X.17.06

Vijayaraghavan, P., Primiya, R., & Vincent, S. (2013). Thermostable alkaline phytase from *Alcaligenes* sp. in improving bioavailability of phosphorus in animal feed: In vitro analysis. *ISRN Biotechnology, 2013*, Article 394305. https://doi.org/10.5402/2013/394305

مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸

تعیین ژنوتیپ ایزوله‌های بالینی Serratia marcescens با استفاده از تکنیک‌های مولکولی

کَظَر انتظار

گروه زیست‌شناسی، دانشکده آموزش، دانشگاه القادسیه، عراق. ایمیل: aintizar.kazar@qu.edu.iq

حسین أزهر

*نویسنده مسئول: گروه زیست‌شناسی، دانشکده آموزش، دانشگاه القادسیه، عراق. ایمیل: azhar.almousawi@qu.edu.iq

تاریخ دریافت: ۱۴۰۳/۱۲/۱۷ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۴/۰۲/۱۶ تاریخ پذیرش: ۱۴۰۴/۰۲/۱۷

چکیده

هدف: Serratia marcescens یک باسیل گرم‌منفی، بی‌هوازی اختیاری و متعلق به خانواده Enterobacteriaceae است. این باکتری یک پاتوژن فرصت‌طلب محسوب می‌شود که به‌دلیل نقش آن در طیف گسترده‌ای از عفونت‌های بیمارستانی و اکتسابی از جامعه، از جمله عفونت‌های دستگاه ادراری، عفونت‌های دستگاه تنفسی، عفونت‌های خونی و عفونت‌های زخم، مورد توجه فزاینده‌ای قرار گرفته است. این باکتری همچنین به‌دلیل توانایی زنده‌ماندن در شرایط محیطی متنوع و مقاومت ذاتی به چندین نوع آنتی‌بیوتیک شناخته می‌شود که درمان عفونت‌های ناشی از آن را در محیط‌های بالینی چالش‌برانگیز می‌کند. ژن 16S rRNA، که در میان باکتری‌ها بسیار محافظت‌شده است، ولی دارای نواحی متغیر نیز می‌باشد، به‌طور گسترده برای مطالعات فیلوژنتیک و شناسایی باکتریایی استفاده می‌شود. تعیین ژنوتیپ با استفاده از آنالیز پلی‌مورفیسم طول قطعات برشی (PCR-RFLP) ژن 16S rRNA امکان تمایز سویه‌های باکتریایی را بر اساس حضور جایگاه‌های برش آنزیمی خاص فراهم می‌سازد. این تکنیک دیدگاه‌هایی در مورد تنوع ژنتیکی و روابط تکاملی بین ایزوله‌ها ارائه می‌دهد. هدف این مطالعه تعیین ژنوتیپ ایزوله‌های بالینی S. marcescens به‌دست‌آمده از منابع مختلف بهداشتی و درمانی در استان دیوانیه، عراق با استفاده از تکنیک‌هایی نظیر PCR، تعیین توالی و PCR-RFLP با آنزیم‌های برشی AluI و MspI و بررسی تنوع ژنتیکی ایزوله‌های محلی بود.

مواد و روش‌ها: در مجموع ۲۰۰ نمونه بالینی از بیماران مراجعه‌کننده به بیمارستان آموزشی دیوانیه و چندین درمانگاه خصوصی در سطح استان دیوانیه عراق جمع‌آوری شد. نمونه‌ها بر روی محیط‌های کشت انتخابی و افتراقی استاندارد کشت داده شدند. DNA ژنومی از ایزوله‌های خالص‌شده S. marcescens با استفاده از کیت تجاری استخراج DNA باکتری استخراج شد. شناسایی مولکولی با تکثیر ژن 16S rRNA با استفاده از پرایمرهای جهانی باکتریایی انجام شد. محصولات PCR ایزوله‌های تأییدشده S.

marcescensخالص‌سازی شده و به یک مرکز تعیین توالی تجاری در کره جنوبی ارسال شدند. درخت‌های فیلوژنتیکی با روش Neighbor-Joining رسم شدند. برای ارزیابی تنوع ژنتیکی در میان ایزوله‌های S. marcescens، آنالیز PCR-RFLP ژن 16S rRNA انجام شد. الگوهای RFLP متمایز تحلیل شدند و تعداد ژنوتیپ‌های مختلف بر اساس الگوی باندی حاصل از هر آنزیم تعیین گردید.

نتایج: در مجموع ۲۰ ایزوله از Serratia marcescens از منابع مختلف به‌دست آمد که شامل ۱۵ ایزوله (۷۵٪) از عفونت‌های دستگاه ادراری، ۳ ایزوله (۱۵٪) از سوختگی و زخم‌ها، و ۲ ایزوله (۱۰٪) از آسیب‌های چشمی بودند.

نتیجه‌گیری: این مطالعه نشان داد که تکنیک‌های مولکولی داده‌های دقیقی در مورد ساختار ژنتیکی Serratia marcescens فراهم می‌کنند که موجب بهبود دقت تشخیص داده شده و مسیر توسعه ابزارهای تشخیصی حساس‌تر و قابل‌اعتمادتر را در آینده هموار می‌سازد.

کلمات کلیدی: تعیین ژنوتیپ، 16srRNA، PCR-RFLP، Serratia marcescens

نوع مقاله: پژوهشی.

استناد: کَظَر انتظار، حسین أزهر (۱۴۰۴). تعیین ژنوتیپ ایزوله‌های بالینی Serratia marcescens با استفاده از تکنیک‌های مولکولی. *مجله بیوتکنولوژی کشاورزی*، ۱۷(۲)، ۲۶۳-۲۸۴.

Publisher: Faculty of Agriculture and Technology Institute of Plant Production, Shahid Bahonar University of Kerman-Iranian Biotechnology Society.