Comparative Phenotypic and Genotypic Properties of Serratia marcescens Isolates from Clinical Cases from human in AL-Diwaniyah Province, Iraq

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KEYWORDS
Phylogenetic tree, Serratia marcescens, prodigiosin, pigM, and burns.

ABSTRACT:
Serratia marcescens is a Gram-negative, rod-shaped bacterium. It is an opportunistic and facultative anaerobe. This study aims to determine the occurrence rates of Serratia marcescens in human clinical samples, investigate the pigM gene, sequence the isolates, and compare them with global isolates documented in the NCBI database. Fifty burn samples were collected from the specialized center for burns in AL-Diwaniyah province, along with 35 urine samples from individuals suffering from UTIs from Al-Diwaniyah Teaching Hospital and various private laboratories. These samples were transferred in a cooled container to the microbiology lab at the Veterinary Medicine College, University of Al-Qadisiyah, for bacteriological assay. The samples were then cultured using selective and differential media. Following the purification of the cultured bacteria, biochemical tests were conducted to confirm the isolates. Results showed that 10 out of 85 samples (11.76%) were positive for Serratia marcescens. Culture positivity was most frequent in urine samples (17.1%), followed by burn samples (8%). Molecular methods revealed that human isolates gave 100% positive results for 16S rRNA. The study found that ten isolates of Serratia marcescens possess the pigM gene, with the 16S rRNA gene sequenced using the BLASTN algorithm of NCBI. All isolates were recorded under accession numbers (OR835629, OR835630, OR835631, OR835632, OR835633, OR835634, OR835635, OR835636, OR835637 and OR835638, showing 97-99% identity with isolates detected in China, India, and Nigeria. It can be concluded that the isolation rates of Serratia marcescens from urine samples are higher than those from burn samples. The sequencing results showed a 97-99% similarity with global isolates from China, India, and Nigeria.

1. Introduction

Serratia marcescens is a rod-shaped, gram-negative, motile, facultatively anaerobic bacterium belonging to yercinayai (1). suggest that *Serratia marcescens* is known to be a prevalent species in clinical settings, responsible for urinary tract infections (UTIs), meningitis, pneumonia, infective endocarditis, catheter-associated bloodstream infections, wound infections, as well as myriad other conditions (3). Additionally, *S. marcescens* can cause osteomyelitis, septicemia, and eye, and respiratory tract infections, with transmission occurring through direct contact (4). The bacterium can proliferate in supposedly sterile fluids and on catheters, as evidenced by an *S. marcescens* outbreak in a Taiwanese hospital linked to contaminated intravenous painkiller fluids (5).

Serratia marcescens is known to harbor multiple drug-resistance genes and is a significant cause of nosocomial infections, along with other members of the Enterobacteriaceae family. Examples of such infections include those originating from burns, UTIs, intravenous line-related bloodstream infections, and infective endocarditis. Strains of *S. marcescens* have been isolated from water supplies, food, and dairy products (6). Despite the fact that *Serratia marcescens* is a prolific producer of phenazine-type antibiotics that have proven to be effective against plant pathogens, there is evidence suggesting that it may also end up exacerbating root rot in various sprout systems. These risks, in conjunction with the challenges posed by its antimicrobial resistance, necessitate a thorough investigation. Therefore, this study employed both phenotypic and genotypic screening methods to obtain contemporary genetic data on *S. marcescens* strains isolated from clinical cases (7). Through this comprehensive approach, we aim to better understand the genetic variations and resistance mechanisms that contribute to the pathogenicity and persistence of *S. marcescens* in different environments.

Serratia marcescens is recognized as a notable opportunistic pathogen, frequently causing nosocomial pneumonia, urinary tract infections, wound infections, bacteremia, and endocarditis. The increasing prevalence of this pathogen has made treatment more challenging due to its heightened antimicrobial resistance (8) Numerous reports have identified extended-spectrum beta-lactamase-producing *S. marcescens*, multi-drug-resistant *S. marcescens* co-expressing multiple carbapenemases, and strains with phenotypic and genotypic

resistance to multiple β-lactamase types, resulting in decreased sensitivity to carbapenems and the production of metallo-β-lactamases, thereby reducing the efficacy of most β-lactamase antibiotics. In this study, nine clinical isolates of *S. marcescens* were collected and analyzed using various experimental methods. High sensitivity rates to ceftazidime, gentamicin, and amikacin were observed, while low sensitivity to cefotaxime, ciprofloxacin, and co-trimoxazole were noted (9).

Natural pigments can be synthesized from microorganisms, and these microbial pigments are valued for their stability and the ease with which they can be cultivated (10,11,12,13,14). These microorganisms grow rapidly in inexpensive culture media, independent of environmental conditions, and produce various shades of colors. Consequently, microbial pigment production is an emerging research field with potential applications across multiple industries (15). The prodigiosin pigment of *Serratia marcescens* and the violacein pigment of *Chromobacterium violaceum* are linked to the cells that produce them and are lipid soluble. Their most notable uses are in pharmacology (16), with prodigiosin being the most extensively researched. Recent studies on the antibacterial activity of prodigiosin have revealed membrane damage as a recurring theme (17).

The biosynthesis of MBC in *Serratia* sp. and *Streptomyces* has been studied using genetic approaches, complementation, and in vitro analysis of the purified enzymes involved in the early stages of this pathway (18). The degree of conservation of the prodiginine biosynthetic clusters suggests that MBC biosynthesis proceeds via a standard route requiring proline, acetate/malonate, serine, and methionine. Proline is incorporated to form pyrrole through a mechanism common to other pyrrole-containing compounds such as chlorobiocin, coumermycin A1, novobiocin, and pyoluteorin. In prodiginine biosynthesis, proline is incorporated to form the pyrrolic ring (12, 13, 19, 20-22).

The current study aims to determine the occurrence rates of *Serratia marcescens* in burn and urine samples in humans, sequence the isolates, and compare them with global isolates documented in the NCBI database.

2. Materials and Methods

Samples collection

Whole of 85 samples, 50 burns samples were collected from the specialized center for burns in AL-Diwaniyah province, and 35 urine samples were collected from persons suffering from UTI from Al-Diwaniyah Teaching Hospital and different private laboratories from March 2021 through August 2021; these samples were transferred to the lab in a cooled container.

Identification of *Serratia marcescens*

Bacterial isolation

Samples were brought to the microbiology lab at the University of Al-Qadisiyah College of Veterinary Medicine for bacteriological testing. All specimens were inoculated in nutrient broth and incubated for 24 hours at 37 degrees Celsius. Subsequently, the surface of MacConkey agar and tryptic soy agar was loop-streaked, with the colonies of *Serratia* spp. appearing small, round, and red on TSA. These colonies were suspected to be red or non-lactose fermenting on MacConkey agar and were subcultured on Orientation Chrom agar for further confirmation on culture media.

Each isolate was fixed as a single colony on a sterile slide to examine the Gram stain response under a light microscope. After the filtration of cultivated microorganisms, the genus and species of the isolated bacteria were confirmed through standard biochemical tests for the isolates from the bacterial colonies (23) , including tests for arabinose and lactose fermentation, catalase, oxidase, urease indole production, Voges-Proskauer reaction, methyl red test ,citrate utilization, motility, esculin hydrolysis, triple sugar iron, and DNase activity.

Confirmation by PCR technique and Measured DNA level and Purity

Extraction of genomic DNA: Using a DNA kit (Geneaid/Taiwan), *S. marcescens* extracted their DNA based on the company's instructions. The extracted DNA was then examined for quality by utilizing a Nanodrop spectrophotometer to detect DNA content (ng/µL) and absorbance at (260 / 280 nm).

Polymerase chain reaction (PCR) and Amplification of Primers

For confirmative detection, a PCR experiment was conducted utilizing primer 3 plus design online and NCBI-GenBank to create the *S. marcescens*-based 16S rRNA gene (24) and the pigM gene Bioneer Company, Korea, supplied the primers as Table (2.1). All primer pairs used in the study, including product size and amplification conditions, were carried out by PCR and are listed below. Each PCR reaction was conducted in a total volume of 50 µl, as detailed in Table 2.2.

Table 2.1. Sequences of primers with size of targeted products of the PCR to the *Serratia marcescens*

Primers		Sequences		Amplicon	GenBank / Reference
1	16S rRNA	F	AGAGTTTGATCCTGGCTCAG	1500 bp	Eden et al.1991
		R	GGTTACCTTGTTACGACTT		
2	pig M	F	TCAATACATCCGCTTCAACGAA	456 bp	This study
		R	AAAATGCTTTGCCAGCTGC		

Table 2.2 PCR Mixture

No	The Contents	V (µl)
1	Master Mix	25
2	F- Primer	3
3	R-Primer	3
4	Sample DNA	5
5	Nuclease	14
Total		50

The following thermocycler setup was used to conduct the reaction in a thermocycler: **a.16rRNA:** 94°C was the starting denaturation temperature for 5 minutes. Subsequently, this was followed by 30 cycles of denaturation for 60 seconds at 94°C, annealing for 60 seconds at 55°C, extension for 90 seconds at 72°C, and final extension for 10 min at 72°C. The PCR products were then evaluated by electrophoresis on a 1% agarose gel.

b. pigM gene: To begin with, a denaturation period of five minutes was established at 94 °C. Subsequently, there were thirty cycles of denaturation for thirty seconds at 94 °C, annealing for thirty seconds at 52 °C, extension for less than a minute at 72 °C, and final extension for ten minutes at 72 °C. The PCR products were run on an agarose gel (1.5%) for the purpose of analysis.

Sequencing

Bacterial Genes Sequencing and Analysis of the Sequencing Results

Macrogen Company (South Korea) sequenced the 16S rRNA PCR products. The samples were supplied to Iraq Biotechnology with the following specifications: 50 µl (10 pmoles/µl) for each forward and reverse primer and 15 µl for each forward and reverse PCR product. The samples were provided in a cool box with an ice pack, and each was tagged with a number and name corresponding to the numbers sent to the firm. The NCBI's BLASTN algorithm was used to analyze the 16S rRNA gene sequencing results for both forward and reverse sequences. The NCBI's Blast Nucleotide Sequence tool was then used to apply the treatment sequence from the text file (http://blast.ncbi.nlm.nih.gov). The percentage of the comparable sequence is shown beside the name of the bacterial species. A set of 16S rRNA sequences with 100% identity, 100% query coverage, and an e-value of 0.00 registered in the NCBI databases is considered an excellent match for the query sequence, according to BLASTN results.

Phylogenetic Tree

The *Serratia marcescens* isolates' phylogenetic tree was constructed using the Rooted Neighbor-Joining method. Concatenated sequences for each strain were used to create this tree. These sequences were obtained through an alignment of 16S rRNA gene sequences, which was generated using the Molecular Evolutionary Genetics Analysis (MEGA) 10 tool. This phylogenetic tree illustrates the geographical and evolutionary relationships between *Serratia marcescens* isolates from the current research and reference strains.

3. Result

Prevalence of Serratia marcescens and culture, biochemical characterization

The isolation of 85 bacterial samples from various sources (50 burn samples and 35 urine samples) revealed that 10 samples were identified as *Serratia marcescens*. Table 1 provides detailed data on the isolation of the pathogenic bacterium *Serratia marcescens* from human samples collected from burn wounds and UTIs. A total of 85 samples were analyzed, divided into two types: burn samples and urine samples.

The positive rate among burn patients was 8% out of the 50 samples. In conclusion, *Serratia marcescens* was found more prevalently in patients with urinary tract infections compared to those with burns, as anticipated. The data indicate higher levels of *Serratia marcescens* in urine samples (17.1%). Among the 35 urine samples collected from patients with suspected UTIs, 6 tested positive for the bacterium, highlighting a higher prevalence in this population compared to the burn samples. This suggests a significant presence of the bacterium in urinary infections, warranting attention from healthcare providers.

Combining data from both types of samples, we analyzed 85 samples, of which 10 were positive for *Serratia marcescens*, resulting in an overall infection rate of 11.76%. Therefore, the aggregated data underscores the need to monitor and control this opportunistic pathogen in healthcare settings, as it proves to be more prevalent in urinary tract infections (Figure 3.1).

In summary, although *Serratia marcescens* is found in both burn wounds and UTIs, its higher frequency in urinary tract infections is more critical and warrants control measures. These findings could aid in developing targeted infection control strategies, potentially improving patient recovery rates in healthcare institutions. Table (3.1) highlighted that urine represented (17.1%), while burns represented (8%).

Table 3.1 Number and Percentage of positive *Serratia marcescens* isolated from Burns and UTI from humans.

No.	Sample type	Number of samples	Positive sample	Percentage
1	**Burns**	50	4	8%
2	**Urine**	35	6	17.1%
Total		85	10	11.76%
X2		1.65		
P value		0.198 (non-significant)		

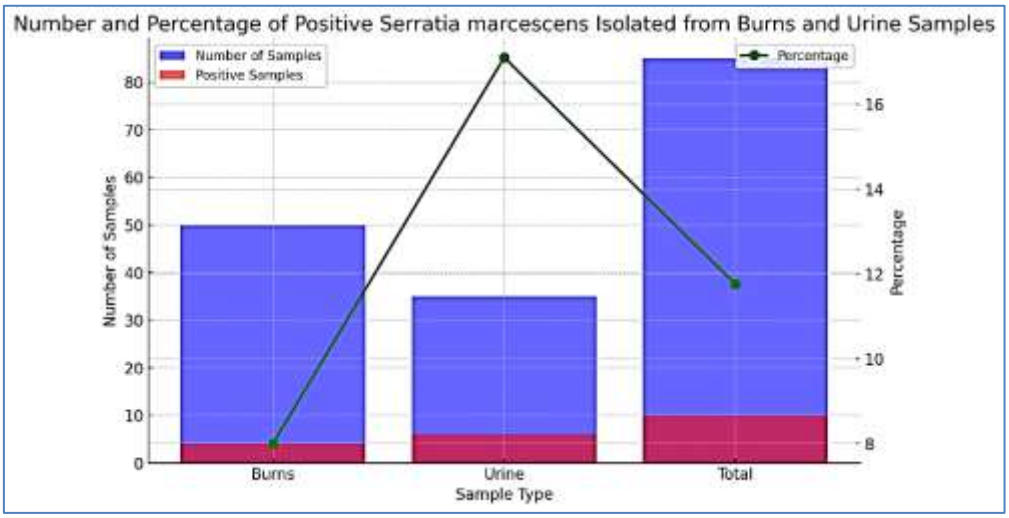

Figure 3.1. Represents sample sources versus the rate of isolation of *Serratia marcescens* from these sources of humans

Morphology and biochemical characterization for *S marcescens*

After incubation at 37°C for 24-48 hours, the study showed that the growth of *S. marcescens* on MacConkey agar at 37°C was identified by comparing the colony morphology small, smooth, pale colonies (non-lactose

fermenting) as shown in Figure 3.2.A. Colonies on MacConkey agar and Tryptic soy agar (TSA) at 25-28°C displayed a red color due to the production of the prodigiosin pigment (Figures 3.2.B, C), and metallic blue growth on CHROM agar Orientation (Figure 3.2.D).S marcescens has fishy–urinary odor ,on blood base agar *S marcescens* give beta hemolysis colonies due to present the (ShlA) is the only hemolysin that has been reported in *S. marcescens* (43).

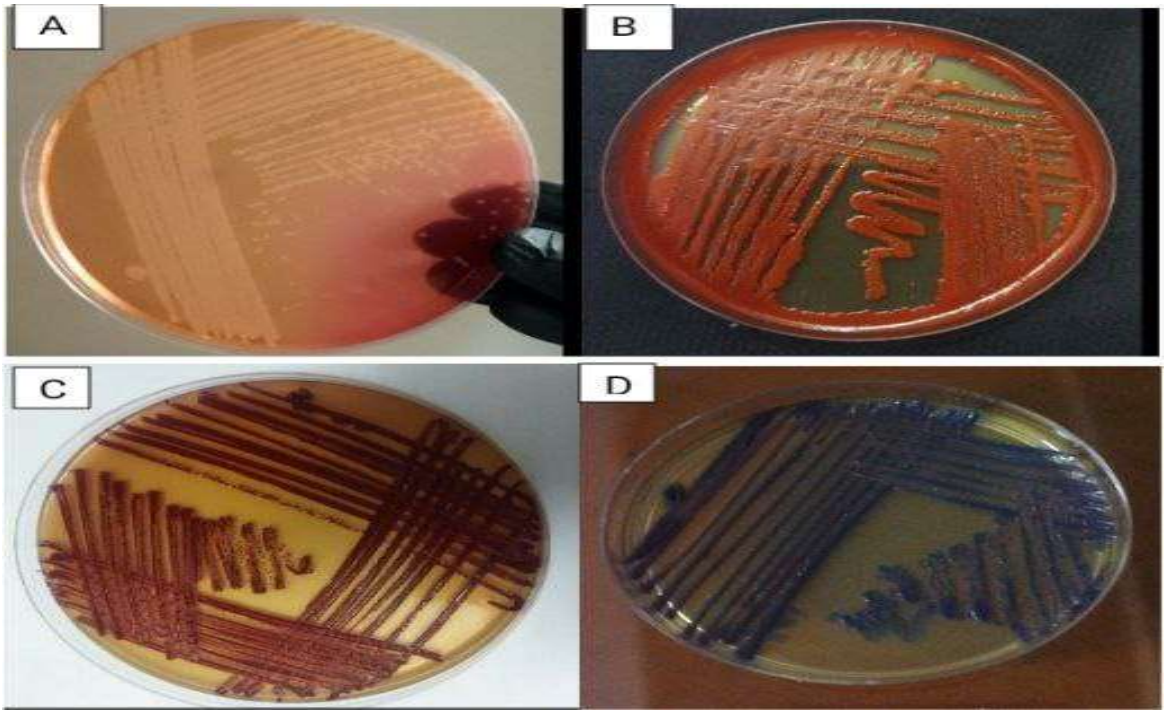

Figure 3.2. Isolation and identification of *Serratia marcescens* from human: A. *Serratia marcescens* colonies on MacConkey agar (Pale). B. *Serratia marcescens* colonies on TSA (red colonies due to produce prodigiosin) .C. *Serratia marcescens* MacConkey agar at 28º C. D. *Serratia marcescens* colonies on CHROM agar Orientation (metalic blue).

Upon Gram staining, all isolates were confirmed as Gram-negative cells. Additionally, all isolates were positive for catalase, DNase, Voges–Proskauer , citrate utilization, Esculin hydrolysis, and motility tests, while negative for indole production, oxidase activity, and arabinose fermentation ,TSR reading K/A ,no gas, no H2S (Table 3.2 and Figure 3.3).

Table 3.2 Biochemical test and staining for *Serratia marcescens* isolates

The biochemical examination and stain		The result
1	Gram stain	Gram-negative rod ,rounded end
2	Indole	-
3	Voges–Proskauer	+
4	Citrate utilization	+
5	Esculin test	+
6	Methyl red test	+
7	Catalase Test	+
8	DNase test	+
9	Oxidase test	-
10	Motility test	+
11	urease	+
12	arabinose fermentation	-
13	TSI test	K/A,no gas, no H2S

+ =positive - = negative K= alkaline A= acid

Molecular confirmation

The molecular detection results revealed that all isolates showed positive findings (lines 1-10) on confirmatory endpoint PCR, which detects the 16S rRNA gene (1500 bp) on an ethidium bromide-stained agarose gel using specific primers and a ladder with sizes ranging from 100 to 3000 bp. The primer sequences, with product sizes of 456 bp, were used to examine ten isolates of *Serratia marcescens* for the presence of the *pigM* gene. All isolates tested positive for the *pigM* gene (Figures 3.4 and 3.5).

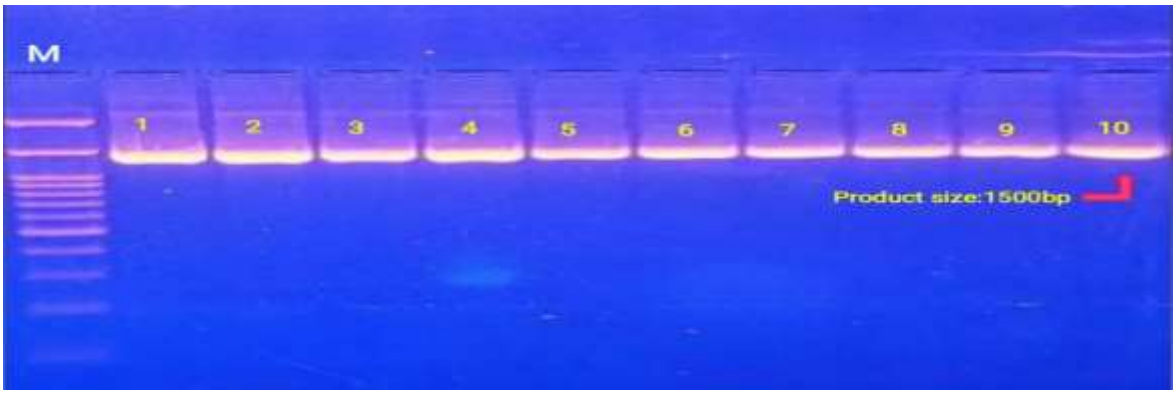

Figure 3.4. PCR product analysis of the 16S r RNA gene in isolates positive for Serratia marcescens, as seen in this Agarose gel electrophoresis picture. Where M is the marker (100–3000 bp), lane (1–10) indicates a positive S. marcescens at the 16S r RNA gene PCR product (1500 bp)

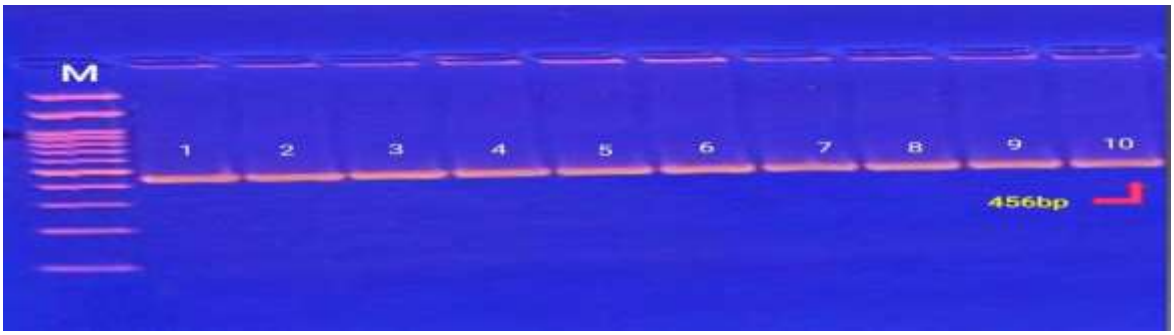

Figure 3.5. A picture from an agarose gel electrophoresis showing the pigM gene's PCR product analysis in isolates that tested positive for Serratia marcescens, Where lane (1–10) positive *S. marcescens* at (456 bp) pigM gene PCR product, and M: marker (100–3000 bp).

Sequencing and phylogenetic tree construction of 16S rRNA gene

Ten *Serratia marcescens* isolates which positive for PCR sent to Korea for sequencing and record in NCBI-GenBank, at accession number codes [OR835629, OR835630, OR835631, OR835632, OR835633, OR835634, OR835635, OR835636, OR835637 and OR835638] the 10 *Serratia marcescens* isolates were aligned with global reference strains for *Serratia marcescens* recorded in the GenBank table: 3.3

Analysis of the Phylogenetic Tree

The tree structure of the phylogenetic tree is rooted. This indicates the evolutionary relationships among different Serratia marcescens strains and each branch point (node) is a common ascendant of the different Serratia marcescens distinguish the source. The length of the different branches can imply streamlined distance or evolutionary time. These strains are illustrated and also labeled (indicated by Plus symbols at the top of the image) with the sequence identifiers and the geographic origins (as indicated by colored bubbles and the texts below them). the sequence identifier and geographical origin are useful to see the distribution of these strains geographically and some migration pathways. These numbers (shown on branch points of the phylogenetic tree) are called bootstrap values. It can indicate the confidence level when collating these strains with a specific branching order (the higher value means this branching order is more confident). Red Circles are also present in these images and they indicate some strains or important nodes. Given that tree structure suggests a close

genetic relationship of strains from the same geographical region often cluster together, the trees can imply two things. First, some geographic regions contained diverse Serratia marcescens strains like China or Iraq, as suggested by the big clusters of these regions. Also, the infrequent dispersed strains (e.g., occur between China and Iraq region) indicate how these strains migrate between these terrains and the interaction between them in genetics..

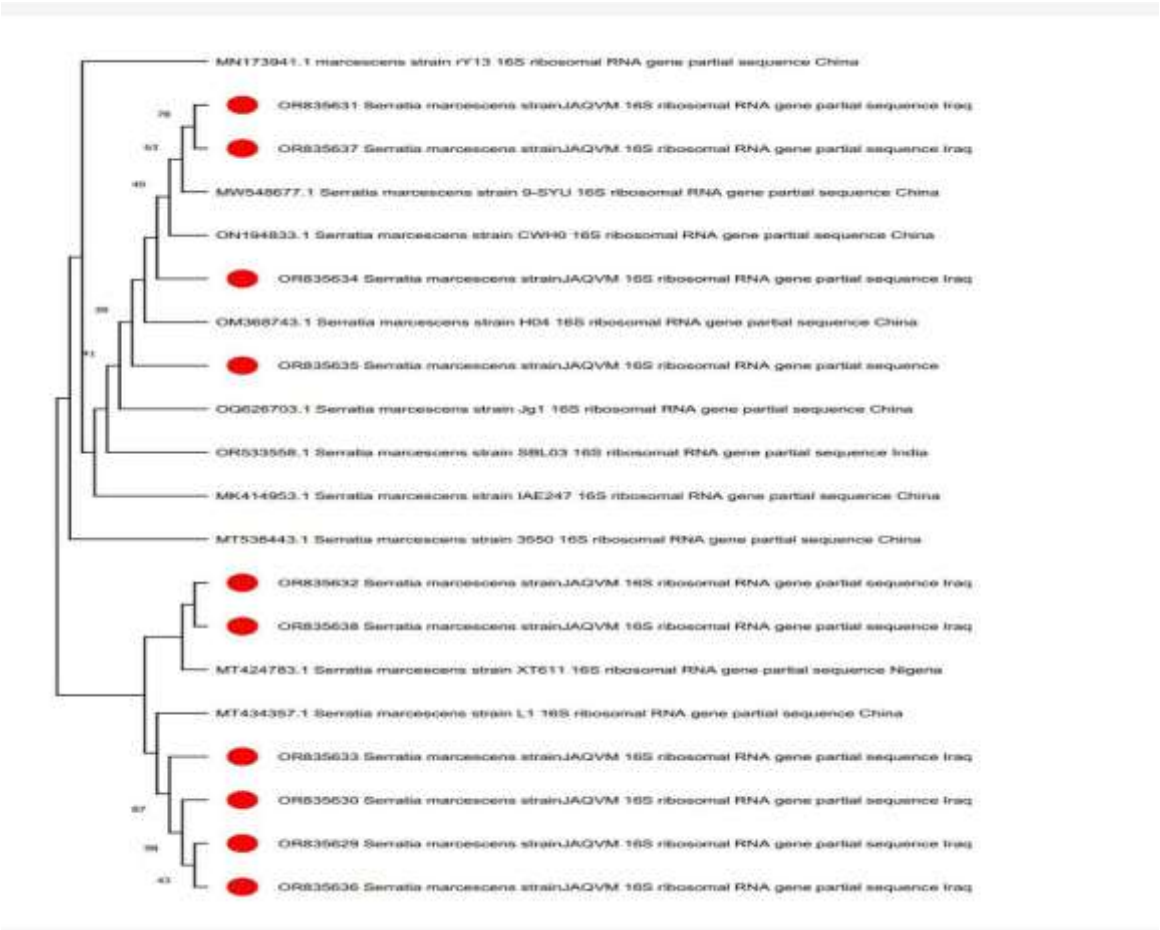

Figure 3.6 Phylogenetic trees of *Serratia marcescens* in human with global isolates

The phylogenetic affiliation of 10 strains of *Serratia marcescens* JAQVM was determined by constructing a phylogenetic tree depicted in Figure 3.6. The phylogenetic tree was constructed using MEGA 11.1 after aligning multiple sequences. Figure 3.7 illustrates the alignment of these ten strains with other closely related 16S rRNA nucleotide sequences derived from BLAST sequence similarity search analysis. Phylogenetic tree analysis revealed that these 10 strains could be categorized into five clades. The first clade includes the following strains: OR835633, OR835630, OR835629, and OR835636. The second clade comprises two strains, OR835632 and OR835638. The third and fourth clades each contain one strain: OR835634 and OR835635, respectively. The fifth clade includes two strains, OR835631 and OR835637.

Table 3.3. NCBI-BLAST Homology sequence Identity (%) in local *S .marcescens*

No.	Accession No for *Serratia marcescens* for this study	Accession No for global isolates	Strain	Country	Identity
1	OR835631 OR835637	MW548677 .1	9-SYU	China	99%
		ON194833.1	CWH0	China	98%
2	OR835634	MW548677 .1	9-SYU	China	99%
		ON194833.1	CWH0	China	97%
		OM368743.1	H04	China	99%
3	OR835635	OQ626703.1	Jg1	China	98%
		OR533558.1	SBL03	China	99%

		MK414953.1	IAE247	India	99%
		OM368743.1	H04	China	98%
4	OR835633	MT434357.1	L1	China	99%
6	OR835636	MT434357.1	L1	China	99%
7	OR835629	MT434357.1	L1	China	98%
8	OR835630	MT434357.1	L1	China	97%
9	OR835632	OR533558.1	SBL03	China	99%
		MK414953.1	IAE247	India	99%
		MT424783.1	XT611	Nigeria	99%
		OM368743.1	H04	China	98%
		MN173941.1	rY13	China	99%
10	OR835638	MT424783.1	XT611	Nigeria	98%
		MT434357.1	L1	China	98%

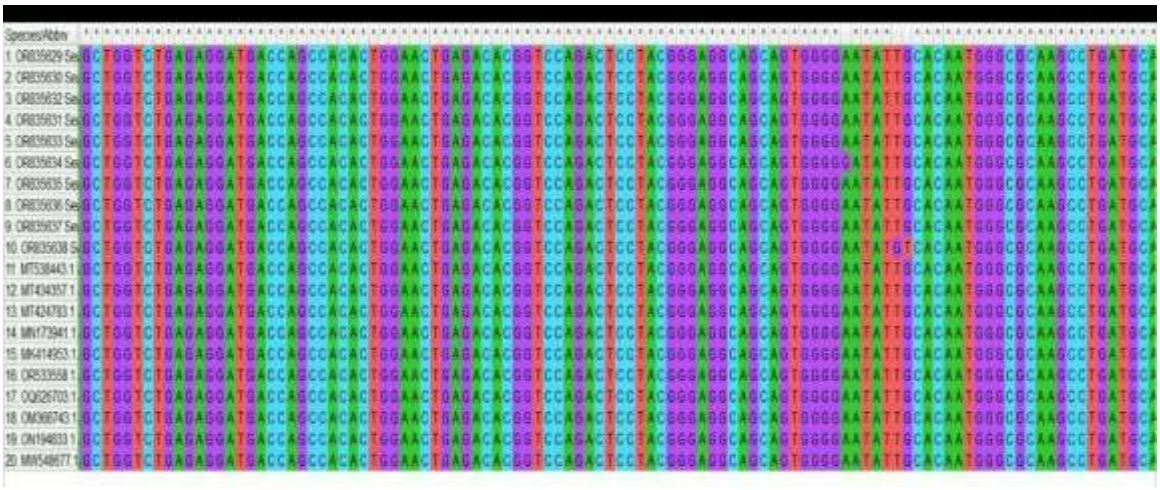

Figure 3.7. Multiple sequence alignment analysis for *Serratia marcescens* compared with homologues global sequence.

Despite the limited discriminatory power of 16S rRNA sequence analysis regarding strain differentiation, the strains could be categorized into five clades as mentioned above. This result reflects the significant genetic diversity among the 10 tested strains. Additionally, the results of the 16S rRNA sequence analysis align well with those obtained using multi-locus sequence typing (MLST) in the prospective study. Moreover, the strain-description highlights the necessity of conducting a large-scale molecular epidemiological study to trace the predominant strains of *Serratia marcescens* implicated in UTI and burn infections among the Iraqi population.

4. Discussion

Isolation rate for S marcescens

S marcescens is known to cause opportunistic and nosocomial infections, affecting individuals across all age groups. Urinary catheters, intubation, and central venous catheters are major entry points for these infections, facilitating bacterial colonization, especially within biofilm communities (25, 26, 27). Various infections caused by Serratia marcescens have been documented, including wound infections, sepsis, pneumonia, meningitis, endocarditis, UTIs and ocular infections (28). Additionally, this pathogen is associated with osteomyelitis and respiratory infections (29).

Our study revealed a higher isolation rate of S. marcescens from urine samples (17.1%) compared to burn samples, which agrees with previous findings by (30). while the isolation rate from UTI in current study was lower than the prevalence rates reported by (31,32,33) who documented higher isolation percentages from human urine samples. However, the current study's rate is close to the isolation percentage recorded by (34) in the Wasit province (12.2%). The detection of Serratia spp. as a pathogen in community and hospital-associated UTIs has been extensively recorded in various studies (35).

The isolation rate *of S. marcescens* from burn samples in our study was 8%, which is similar to the findings of (30) who reported an isolation rate of 9.09%. (36) documented an 11% isolation rate from burn wound infections. However, our results are lower than those reported by (37) , who recorded a 19.5% isolation rate. The variations in isolation percentages across different studies can be attributed to differences in sample size, study conditions, and geographical factors influencing the *S. marcescens* population.

Morphology and biochemical characterization for *S marcescens* isolation

Colonies of *Serratia marcescens* at 37ºC, 24 h On MacConkey agar appeared small ,pale and rounded (non-lactose fermenting), consistent with previous findings (38,39,40,41) . At 28ºC, colonies displayed a dark red color on the surface of TSA and MacConkey agar due to prodigiosin pigment production, aligning with observations from other studies (42) . On blood agar Colonies of *S marcescens* give hemolysis type beta this result agreement with (43)All isolates tested positive for DNase, Voges-Proskauer, citrate utilization, motility, and catalase tests, corroborating results from other researchers Conversely, all isolates were negative for oxidase, hydrogen sulfide production, arabinose and lactose fermentation. (30 ,37). isolates has fishy-urine odor this result agreement with (44) .

Genomic DNA extraction of *S. marcescens and investigation about the pigM gene*

Genomic DNA was extracted from bacterial isolates using a commercial genomic DNA purification kit (Geneaid/Taiwan) in a short time. All isolates showed bands, which indicated the genomic DNA on agarose gel electrophoresis.as seen using 16S RNA gene, which amplified a product size of approximately 1500 bp , All isolates have pigM , in *S marcescens* pigM gene is responsible for synthesizing a red pigment known as prodigiosin. The pigM gene is part of a cluster consisting of 14 genes necessary for the synthesis of prodigiosin (45,46). (47) suggest that the pigM gene plays a significant role in the overall process of prodigiosin production. The synthesis of prodigiosin involves multiple factors that activate the expression of the pig genes, including pigM (48) .

The production of prodigiosin relies on fourteen genes (pigA-N) that form the pig gene cluster. This gene cluster is observed in selected isolates such as *S. marcescens*, *S. rubidaea*, and *S. plymuthica* (45) . A comprehensive study conducted in a large Brazilian hospital revealed the presence of 54 *S. marcescens* strains among the patients (49) . (48) did not report the number of repetitive reads for the pigM gene, as their study focused on highly expressed genes in lactose-fermenting spontaneous mutants of *S. marcescens*. This highlights the unique features of each research, leading to variations in the detection of the pigM gene among *S. marcescens* isolates, the context of this study, pigM genes were identified in all *S. marcescens* isolates .

Sequence analysis for S marcescens

Our sequencing results demonstrate a genetic identity of 97-99% with international isolates from China and Nigeria. This finding suggests that genetically identical strains of *Serratia marcescens* are prevalent across various regions, indicating high genetic conformity between our sequenced isolates and those from other geographical areas (45) . Understanding the phylogeny and genetic diversity of *S. marcescens* is crucial for comprehending its epidemiology and global distribution. Characterization of isolates from different locations underscores the significance of this knowledge (49.450) .

The phylogenetic tree demonstrates that strains of *Serratia marcescens* sampled from various geographic regions form distinct clusters with notable branch lengths. This prominent clustering is particularly evident among Chinese and Iraqi strains. Such patterns likely arise because strains from the same region tend to be more genetically similar due to localized evolution and limited genetic exchange with strains from other regions. Geographic isolation often results in the evolution of different genetic lineages, driven by the absence of gene flow and unique selective pressures within distinct environments (51) ,in addition the sememlarity between our study strains and Chinese strain may due to the large number of medical supplies manufactured in China, and because S. marcescens is opportunistic and exploits primary infections for UTIs which can also transit through these supplies.(52) while the similarity with Indian strain may attribute to the fact that most patients choose to travel to India for treatment .

Red circles in the phylogeny highlight specific strains or nodes of interest, potentially indicating clinical relevance or aparticular evolutionary branch within the gene tree. It is important to consider the potential

human impacts of these strains: Which strains have been linked to hospital-acquired infections? What antibiotic-resistance genes do they carry? Which strains exhibit such a divergent gene pool that they are placed on different branches of the tree, suggesting they evolved in separate environments? For example, *Serratia marcescens* is renowned for its capability to acquire antibiotic resistance mechanisms and cause various hospital-acquired infections (53)

Understanding the clustering and divergence within the phylogenetic tree can shed light on the epidemiology and spread of *Serratia marcescens*. Strains with clinical significance, such as those associated with hospital outbreaks or carrying resistance genes, can be identified and monitored. Furthermore, the genetic diversity illustrated in the tree underscores the importance of regional surveillance and tailored infection control measures. The evolutionary mechanisms driving this diversity, including horizontal gene transfer, mutation, and selective pressures, are critical for predicting and managing the emergence of new, potentially more virulent or resistant strains of *Serratia marcescens*.

In a similar vein, the bootstrap values (e.g., 78, 63, 49) on the branches estimate the confidence level for these branching orders. Bootstrapping quantifies the support for the inferred tree: if the data is resampled multiple times and the tree is recalculated, any branch with a high value will consistently appear. This method for demonstrating robustness in phylogenetics was introduced by David S. Felsenstein in 1985. Generally, a value of 70 percent or above is considered to provide strong support for a particular branch (54) .

A more complex phylogenetic analysis of the *Serratia marcescens* strains involved in human infections has significant applications for public health and epidemiology. Firstly, it can enhance the understanding and tracking of infection spread, thereby guiding specific control measures. The clustering of strains from regions such as China or Iraq might indicate a regional outbreak or the endemic presence of specific lineages, informing local interventions (55) .

The genetic diversity observed in the phylogenetic tree offers insights into the evolutionary mechanisms underlying *Serratia*'s adaptation to various ecological niches. Horizontal gene transfer, mutation, selective pressures from the host environment, and antibiotic usage are likely contributors to the observed variability (56) . Understanding these evolutionary mechanisms allows us to predict the emergence of new *Serratia* strains and their potential impact on human health (4).

5. Conclusion

In the current study, eighty-five samples were collected from a specialized center for burns, along with thirty-five urine samples from individuals suffering from UTIs at Al-Diwaniyah Teaching Hospital. Using conventional methods Serratia marcescens 10 was isolated from these human cases the prevelans rate from urine was higher than recorded in burns samples. Molecular techniques confirmed positive results in all isolates (10) of the isolates. This study indicated that all isolates of *Serratia marcescens* carrying the pigM gene. At the same time, these isolates exhibited 97-99% homology with those reported in China , Nigeria and India

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