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PHENOTYPIC AND GENOTYPIC DETECTION *OF SERRATIA MARCESCENS* EFFLUX PUMPS, BIOFILMS FORMATION, AND ANTIMICROBIAL RESISTANCE PROPERTIES IN DIFFERENT CLINICAL CASES IN WASIT PROVINCE, IRAQ

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Abstract

Serratia marcescens Antibiotic-resistant bacteria with efflux pumps and biofilm formation, posing significant challenges in infection management. In the current study, 18 *Serratia marcescens* isolates were tested for antibiotic susceptibility and the reality resistance mechanisms. The isolates were obtained from urinary tract, wound, and respiratory tract infections.Phenotypicdetection methods, including efflux pumps of the Ethidium bromide-agar cartwheel method and biofilm formation assays, were used. Genotypic detection involved amplification of efflux pump and biofilm formation genes PCR assay using primersspecific for(efflux pumps genes*SdeAB-B, SdeCDE-D, SdeXY-Y*, and *SsmE,*and biofilm genes *YmgB, BsmA*, and *HmsP)*.Gene sequencing was performed and analyzed using BLAST in NCBI and Geneious version -10, revealing similarities between Iraqi *S. marcescens* and global strains. The results showed that *Serratia marcescens* isolates exhibited high resistance to AMC (91.59%), CFM (90%), and CTX (87.48%). while they had the lowest resistance to IPM (35%), CIP (25%), and MEM (20%). Phenotypic detection revealed that (77.7%) of the isolates tested positive for efflux pump activity, while all 18 samples tested positive for biofilm formation using both the microtiter plate (MTP) and modified Congo red agar (MCRA) methods. Genotypic detection demonstrated the presence of *SdeAB-B, SdeCDE-D*, and*SsmE* (100%), *SdeXY-Y* (83.33%), genes associated with efflux pumps. For biofilm formation genes, *YmgB*was present in (94.44%), *BsmA*in (83.33%), and *HmsP* in (77.77%) of the samples.In conclusion, e fflux pumps and biofilm formation contribute to *Serratia marcescens'* resistance to antibiotics. Sequencing revealed novel mutations in these genes, potentially impacting bacterial resistance levels. The appearance of these results brought us beyond the currently documented mutations in NCBI.

Keywords: Serratia marcescens; Antibiotic resistance; efflux pump; Biofilm formation

Introduction

Serratia marcescens is a Gram-negative bacterium that belongs to the Enterobacteriaceae family. It is widely distributed in the environment and has been found in diverse habitats such as soil, water, plants, and animals. *Serratia marcescens* is known for its distinctive red pigment production (Mohajerani et al., 2019). The rise in the incidence of *S. marcescens* infections can be attributed to its multidrug resistance against different classes of antibiotics. This resistance mechanism is facilitated through various pathways. One of the mechanisms involved in antibiotic resistance in *S. marcescens* is the formation of biofilms. Biofilms provide a protective environment for bacteria

(Abbas and Hegazy, 2020). allowing them to resist the effects of antibiotics. Several mechanisms are involved in antibiotic resistance in addition to biofilms, including efflux pumps (Du, et al., 2018). modification of pores in the outer membrane changes in target sites of antibiotic binding, modification of metabolic pathways, and the production of beta-lactamase, extended-spectrum beta-lactamase, and metallo-beta-lactamase. Efflux pump mechanisms enable bacteria to remove antibiotics from inside the cell to the outside, reducing the accumulation of drugs and increasing resistance against antibiotics (Blanco et al., 2016). The expression of efflux pump systems is regulated through various mechanisms, with one of the major mechanisms being the nodulation-cell division (RND)-type efflux pumps ,the presence of an RND family multidrug efflux pump, *SdeAB_B, SdeCDE-D*, and *SdeXY-Y*genes that can pump out a diverse range of substrates that include fluoroquinolones, Chloroma- phenicol, detergent, ethidium bromide, and organic solvents. (Hassan, et al., 2018).Efflux pumps are transporter proteins, located in the cell membrane of Gram-negative and Gram-positive bacteria and the eukaryotic organisms (AL-Ubaidy, 2015). There are six main families of efflux pumps; major facilitator superfamily (MFS), multidrug and toxic efflux (MATE), resistance-nodulation--division (RND), small multidrug resistance (SMR) and ATP binding cassette (ABC), and PACE (proteobacterial antimicrobial compound efflux) family.(Blanco et al., 2016; Du et al., 2018). Depending on the three criteria; substrate specificity, transporters depend on the source of energy, which are either primary active transporters (ATP hydrolysis) as in the (ABC) transporters or secondary active transporters (Na+\H+ pumps) as in the (MATE) transporters (Olivares et al, 2013; Blair et al, 2014) and proton-motive force (PMF) as passive transporters in the (MFS), (SMR) and (RND) pumps (Blanco et al, 2014; Anes et al, 2015). And depending on the phylogenic relationship, which classified as chromosomal or plasmid efflux pumps which genetic code carried on plasmids, transposons , or integrons (Wang et al, 2011; Zhi Li et al, 2016).

Efflux pumps have been recorded as one of the mechanisms responsible for antibiotic resistance in the structures of biofilm. The genes that encode an efflux pump *SdeAB-B, SdeCDE-D,* and SdeXY-Y efflux system of the RND family is the most common efflux system in *S.marcescens*, and *SsmE*gene, which encodes types of MDR-type efflux pumps, are up-regulated undergrowth in biofilms and exposure to different antibiotics. These efflux pumps play a significant role in the bacterium's multidrug resistance against antibiotics (Kumar and Worobec., 2005; Begic and Worobec., 2008; Chen et al., 2003).

The *SdeAB-B, SdeCDE-D, SdeXY-Y,* and *SsmE* system can export Beta-lactams, chloramphenicol, fluoroquinolones, fusidic and fumaric acids, rifampicin, tetracycline, novobiocin, crystal violet, ethidium bromide, bile salts etc (Soto, 2013; Blair et al, 2014). The genome of *Serratia marcescens* contains various resistance genes, including *SdeAB-B, SdeCDE-D,* and *SdeXY-Y,* which encode different types of RND-type efflux pumps. These efflux pumps play a significant role in the bacterium's multidrug resistance against antibiotics and the *SsmE* gene, which encode types of MDR-type efflux pumps (Kumar and Worobec., 2005; Begic and Worobec., 2008; Chen et al., 2003).

This study aims to evaluate mutations in the nucleotide sequences of efflux pump genes, specifically the *SdeAB-B, SdeCDE-D, SdeXY-Y,* and *SsmE* genes, in multidrug-resistant *S. marcescens.* Additionally, the study aims to explore the association of these efflux pump genes with biofilm formation by examining the *HmsP, YmgA,* and *BsmA* genes that are needed to increase *S. marcescens* biofilm production (Kumar and Worobec, 2005; Srinivasan, et al., 2017).

Materials and methods

Bacterial isolates

Eighteen isolates of *Serratia marcescens* were selected for this study(Salim, et al., 2023). , sixteen isolates from urinary tract infection (UTI), two isolates from wound infection, and one isolate from respiratory tract infection(RTI). this study was conducted in the Department of Microbiology aid the faculty of Medicine University of Wasit.

Antimicrobial susceptibility

At first, the disk diffusion method on Muller-Hinton agar, according to the CLSI (2022) guidelines, the susceptibility pattern of isolates was determined against Amoxicillin/Clavulanic acid (30 µg), Cefixime (5 µg), Cefotaxime (5µg), Azithromycin (15 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Meropenem (10 µg), Piperacillin(100 µg), Gentamicin (10 µg), Imipenem(10µg). Then the results by using VITEK®.

Phenotypic detection of efflux pumps S. marcescens

Adopting the method of cartwheel agar-EtBr, using the medium of tryptone soy agar and ethidium bromide dye in different concentrations according to mentioned in (Martins, et al 2013). Various concentrations of ethidium bromidedye (5, 10, 15, 20, 25) (µg/ml) were prepared by adding to the medium of the tryptone soy agar after sterilization and cooling it,The media was shaken well, and after sterilization, it was poured into sterile dishes that were previously divided radially and stored in a refrigerator temperature until use, The bacterial suspension is then swabbed on the EtBr-TSA plates starting from the center of the plate to the edge margin. The dishes were then incubated at 37 °C for 16 hours,The plates were examined under UV light. The fluorescence of isolates at different concentrations of EtBr was noted. Isolates without fluorescence indicated active effluxpumpactivity while those thatfluoresced lacked.

Phenotypic detection of biofilm formation in S. marcescens

A-Micro-titer plate method

Examined the isolates' capacity to generate biofilm on 96-well flat-bottomed micro-titer polystyrene plates. Add 200µL of bacterial suspensions to BHIB for each isolate in three microtiter plate wells. Following that, all microtiter plates were incubated for 24 hours at 37°C. The controls consisted of wells that were stuffed with BHIB. To get rid of the planktonic bacteria, each well's contents were discarded and rinsed three times with phosphate-buffered saline. After fixing the adhesive bacteria with 250 µl (96% ethanol) for 5 minutes, the plates were drained and permitted to dry. We use 100µl of a 1% crystal violet solution (w/v) to stain the plates and then wait 5 minutes. With sterile distilled water, the excess stain was cleaned. By adding 200µl of glacial acetic acid (33%) per well (v/v), microtiter plates were incubated for 15 minutes, and the quantitative analysis

of biofilm was completed(Christensen. et al., 1985).

B-Congo Red Agar medium

A specially prepared medium called Congo Red Agar (CRA) is utilized for this test. *S. marcescens* isolates were inoculated onto CRA and incubated at 37°C for 24 hours. Readings were taken after 24 hours and again after 48 hours. A positive result was indicated by the presence of black colonies with a black crystalline morphology. Non-biofilm producers mostly exhibited colonies that were pink or red (Tahmourespour and Kermanshahi, 2011; Hassan et al.,2011).

Molecular detection

A. DNA extraction identification of efflux pump genes and biofilm genes

According to the manufacturer's instructions. Using specific primers for molecular detection of the efflux pump genes andbiofilm genes for S. marcescens (Table 1) the target genes of interest were amplified using appropriate oligonucleotides (primers) with specific sequences that correspond to the target genes. The PCR-Master mix for each sample was prepared at a total volume of 25µl. The tubes of the mastermix were mixed well using the vortex and subjected to thermos cycler conditions (Table 2). The annealing temperatures required for theamplification reactions were determined based on Table 3

Table 1. Primers used for molecular diagnosis Efflux pump genes and Biofilm genes

Genes	Primer Sequences (5′-3′)		Product Size	Reference
SdeAB-B	F	TCCTCTTATCAGGCCAACGTG	415bp	This study
Efflux pump	R	CGTTCGGCAACAGCTTATCG		
SdeCDE-D	F	CTCGCGCTACATCGAGAAAG	417bp	This study
Efflux pump	R	GGGATCAGCAGATACAGCAAT		
Sdexy-y	F	ATGCTCAAACCGATCCCGAA	488bp	This study
Efflux pump	R	TTAATCTGCGAGAAATGCGCC		
SsmE	F	TGTCGATGGTGGTCAAGAGC	188bp	This study
Efflux pump	R	CACCGAGGCTTTCGACAGTA		
HmsP Biofilm	F	GACGCAGGAGAGCGAGATTT	450bp	This study
	R	GACAATCCCAGATCGTGCAAC		
YmgB Biofilm	F	ATGCCAGCATCGGCTCATT	182bp	This study
	R	AGTGCGACGAAACGTTGCT		
*BsmA*biofilm	F	ATGACGCTTGTCCGCACACT	309bp	This study
	R	TTACCGATACAGAATCGCCTGG		

* A. adenine, C. cytosine, G. guanine, T. Thymine.

Table 2. Preparation of the PCR -Master mix for each sample

Component	Volume / reaction
Mastermix	12.5
DNA template 5-50ng	5
Forward primer (10pmol)	1

Reveres primer(10pmol)	1
Free nuclease water	5.5
Total volume	25

Table 3. PCR Amplification Programs for This Study Bacteria

Gene	**Cycle**				
	1	**35**			**1**
	Initial denaturation	Denaturation	Annealing	Extension	Final extension
Efflux pumps *SdeAB-B*	94ºCfor 1min	94ºCfor 1 min	60ºC for 1 min	72°Cfor 1 min	72ºCfor 1.5min
Efflux pumps *SdeCDE*-D	94ºCfor 1 min	94ºCfor 1min	58ºC for 1min	72ºCfor 1min	72°Cfor 1.5 min
Efflux pumps *Sdexy-y*	94ºC for 1 min	94ºCfor 1min	60ºC for 1 min	72ºCfor 1min	72ºCfor 1.5 min
Efflux pumps *SsmE*	94ºCfor 1min	94ºCfor 1min	60ºC for 1 min	72ºCfor 1min	72ºCfor 1.5 min
Biofilm formation *Hmsp*	94ºCfor 1 min	94ºCfor 1min	60ºC fo r 1min	72ºCfor 1min	72ºCfor 1.5min
Biofilm formation YmgB	94ºCfor 1 min	94ºCfor 1min	60-61ºC for 1min	72ºCfor 1min	72ºCfor 1.5min
Biofilm formation *BsmA*	94ºCfor 1 min	94ºCfor 1min	60-63ºC for 1min	72ºCfor 1min	72ºCfor 1.5min

B.Sequencing Genes

After finishing the PCR and acquiring the PCR products for efflux pump genes and biofilm genes, they were sent to a Macrogen company for sequencing (Macrogen, 2021). The forward and reverse sequences of efflux pump genes and biofilm genes were created for each one. The BLASTN 2.6.1+ program was used to blast the DNA sequence data with the NCBI Ref. sequences database.

C. Mutations in sequences of efflux pumps genes and biofilm genes

Eighteen isolates of Serratia marcescens isolates were selected and nucleotide sequences of target genes were detected by using a sequencer in South Korea (Macrogen company).Thereafter, Geneious program version -10 was used to identify mutations in target genes. Later, the mutations detected in the sequence of these efflux pump genes and biofilm genes were compared with those detected in *S. marcescens* in other countries provided in NCBI.

Statistical Analysis

To conduct the statistical analyses, version 10 of the Statistical Analysis System (SAS, 2014) was utilized. Also, to analyze the differences in percentages, a Chi-square test was carried out. P 0.05 is considered to be accept acceptable significance threshold in statistics.

Results

Antimicrobial susceptibility pattern

The results showed that all isolates of *S. marcescens* were Resistant to class ⎍ - lactam present in class (Amoxicillin + Clavulanic acid AMC) (91.59%), Cefalothin (CFM 90%), Cefotaxime CTX (87.48%), Piperacillin PRL(77.78%), Azithromycin AZM 50.26%), Gentamicin CN (45.44%), Ofloxacin OFX (40.03%), Imipenem IPM, ciprofloxacin CIP and Meropenem MEM, (35%%) (25%) (20%).Show Figure (1) Prevalence of antibiotic resistance among *S. marcescens* isolates.

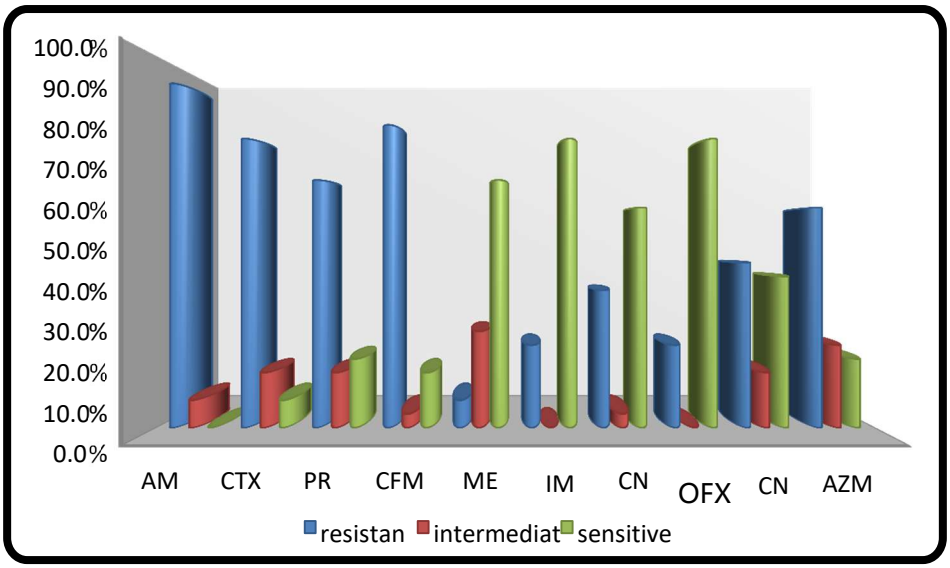

Figure 1. Resistance spectrum of *Serratia marcescens*to antibiotics

Phenotype detection to efflux pumps in Serratia marcescens

Efflux pumps of the Serratia marcescensisolates in method cartwheel agar-EtBr.The results showed that 14 (77.7%) of the bacterial isolates were positive for phenotypic detection. This is based on the lowest concentration of ethidium bromide dye (5mg/ml) at which the isolates did not appear to fluoresce under the ultraviolet (UV) source as shown in Figure (2).

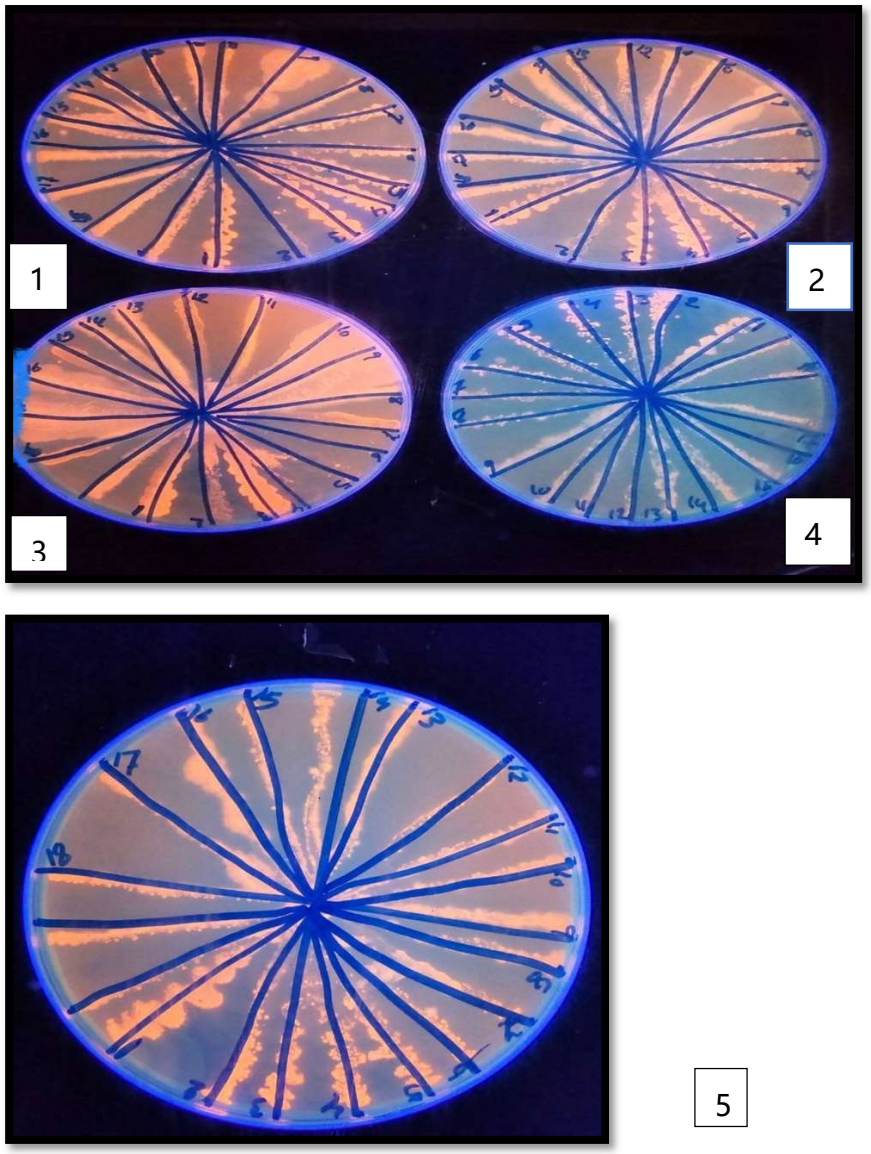

Figure (2): Isolates of non-fluorescent *Serratia marcescens* bacteria in different concentrations of ethidium bromide dye under ultraviolet rays Violet in an experiment with the phenotypic detection of efflux pumps using the agar-ethidium bromide wooden wheel method Tryptone soy agar medium contains *Serratia marcescens* bacteria

Phenotyping detection of Biofilm formation inSerratia marcescens

A-Biofilm formation of the S. marcescens isolates in the MTP method

The results demonstrated that isolates of *Serratia marcescens*had 18 (100%) biofilm production(16(88.88%) isolates produced weak biofilm while (2(11.11%) isolates as moderate biofilm formation).

Biofilm formation of the S. marcescensisolates in the MCRA method

Therewas observations of the colors exhibited by bacterial colony growth on the surface of the

Congo Red Agar plate after (48) hours. The results showed that 18 (100%) were black isolates of *Serratia marcescens* (weak producer biofilm).

Table 4. Distribution of Biofilms Producing *Serratia marcescens* by MCRA vs MTP

Sample	Total No.		positive isolates
		No.	%
samples on the Congo red agar	18	18	100%
Samples on the microtiter plate	18	18	100%
Total	18	18	100%

DNA Extraction, Polymerase Chain Reaction (PCR), Amplification

The bacterial Genomic DNA was extracted using the Genomic extraction kit (Geneaid kit). The extracted DNA showed favorable results. Using a Nanodrop 1000 spectrophotometer set to 260/280 nm, the concentration and purity of DNA are measured. DNA concentration was 180 ng/µl, with a purity of 1.8. Using the electrophoresis (1% agarose, 1X TBE buffer, 70 volts\cm for 60 min.), the amount of DNA extracted from *S.marcescens* transmitted in the gel is 3µl mixed with 2µl of loading dye in each well.

The amplified products of efflux pump genes were run on agarose gel electrophoresis and were found in most isolates of S. marcescens. The product sizes of *SdeAB-B, SdeCDE-D SdeXY-Y,*and*SsmE* genes were about (415 bp, 417 bp, 488bp, and 188bp), Molecular identification of biofilm formation showed the product size The product size of*HmsP,BsmA*, and*YmgB*genes were about (450bp,309bp,and 182bp)as shown in Figure: 4.5.6.7.8.9

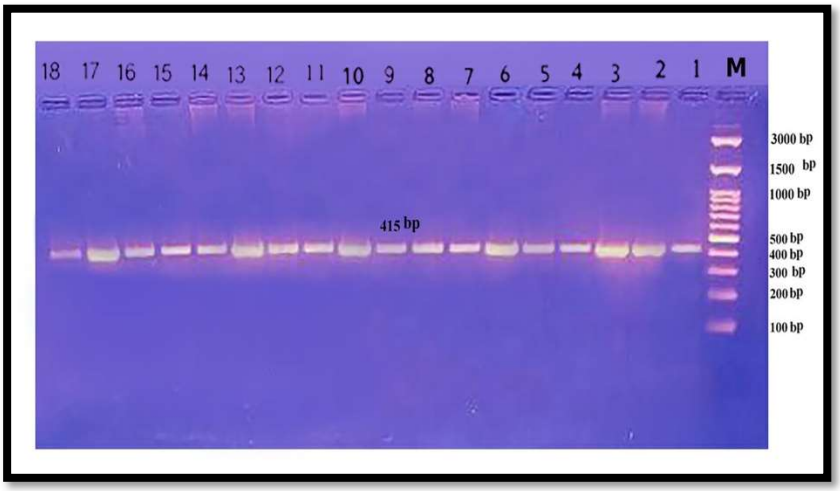

Figure (3) Gel electrophoresis of conventional PCR, the *SdeAB-B*gene from Efflux pump *Serratia marcescens*was amplified and then electrophoresis. Agarose1.5%, 80V/cm for 45 min, ethidium bromide dye staining, and UV transilluminator visualization. M: Marker ladder (100 -3000 bp). Lane (1–18)

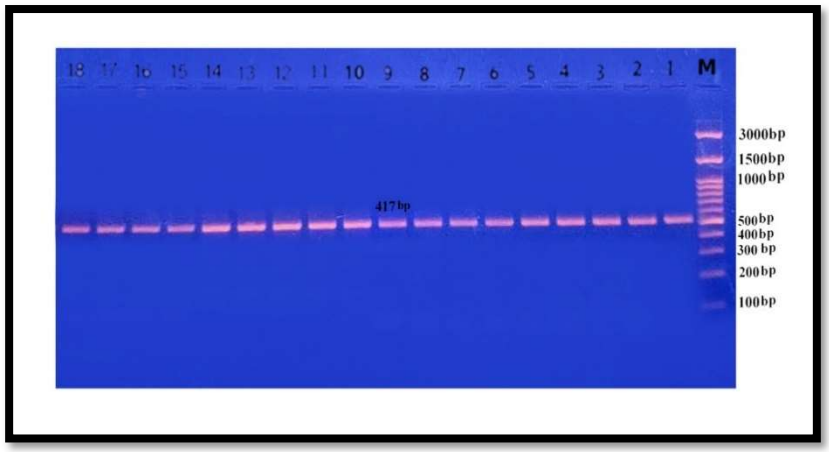

Figure (4) Gel electrophoresis of conventional PCR, the *SdeCDE-D* gene from Efflux pump *Serratia marcescens*was amplified and then electrophoresis. Agarose1.5%, 80 V/cm for 45 min, ethidium bromide dye staining, and UV transilluminator visualization. M: Marker ladder (100 -3000 bp). Lane (1–18)

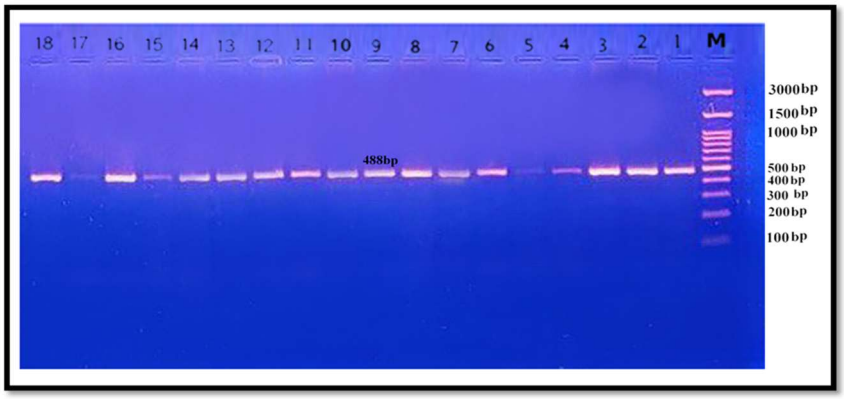

Figure (5) Gel electrophoresis of conventional PCR, the *SdeXY-Y* gene from Efflux pump *Serratia marcescens* was amplified and then electrophoresis. Agarose1.5%, 80 V/cm for 45 min, ethidium bromide dye staining and UV transilluminator visualization. M: Marker ladder (100 -3000 bp). Lane (1–18)

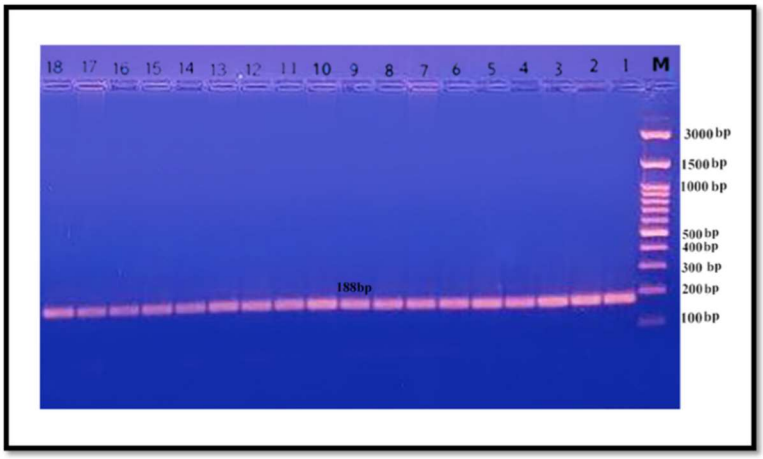

Figure 6. Gel electrophoresis of conventional PCR, the *SsmE*gene from Efflux pump *Serratia marcescens* was amplified and then electrophoresis. Agarose1.5%, 80 V/cm for 45 min, ethidium bromide dye staining, and UV transilluminator visualization. M: Marker ladder (100 -3000 bp). Lane (1–18)

Figure 7. Gel electrophoresis of conventional PCR, the *Hmsp*gene from biofilm *S. marcescens* was amplified and then electrophoresis. Agarose1.5%, 80 V/cm for 45 min, ethidium bromide dye staining, and UV transilluminator visualization. M: Marker ladder (100 -3000 bp). Lane (1–18)

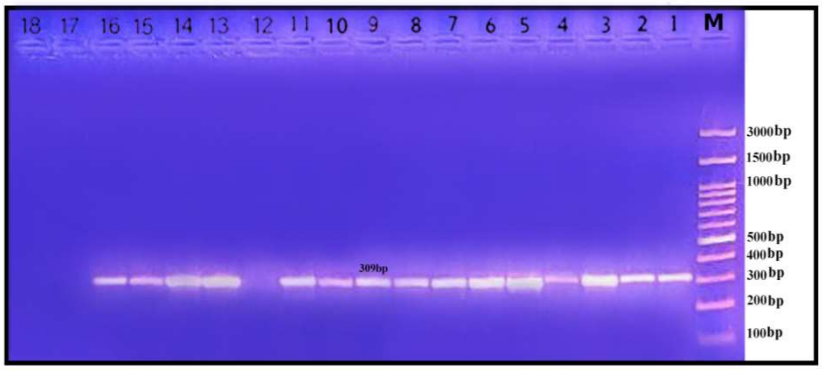

Figure 8. Gel electrophoresis of conventional PCR, the *BsmA* gene from, Biofilm *Serratia marcescens* was amplified and then electrophoresis. Agarose1.5%, 80 V/cm for 45 min, ethidium bromide dye staining, and UV transilluminator visualization. M: Marker ladder (100 -3000 bp). Lane (1–18)

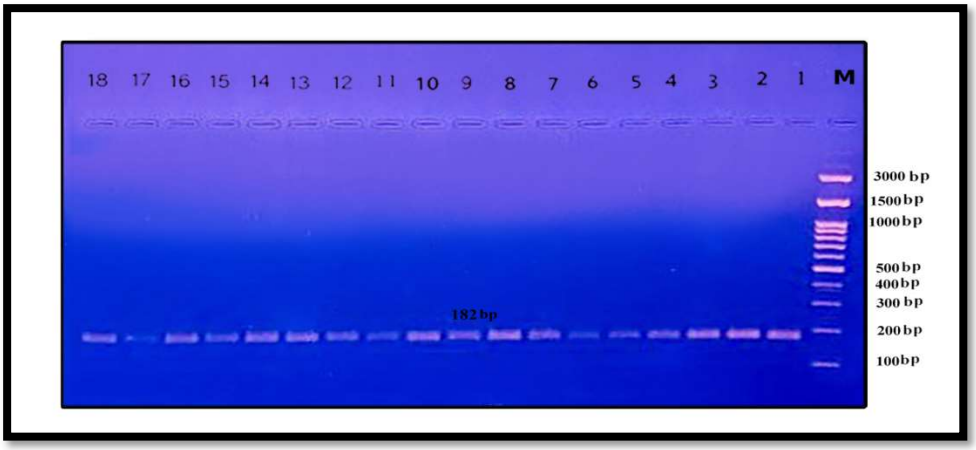

Figure 9. Gel electrophoresis of conventional PCR, the YmgBgene from biofilm *Serratia marcescens* was amplified and then electrophoresis. Agarose1.5%, 80 V/cm for 45 min, ethidium bromide dye staining, and UV transilluminator visualization. M: Marker ladder (100 -3000 bp). Lane (1–18).The relationship between Biofilm Genes Serratia marcescens isolates and Efflux pump genes. As shown in the Table (5)

Table 5. the association between Biofilm Genes *Serratia marcescens* isolates and Efflux pump genes

Virulence Gene	Efflux pump Producer (N=18) n%	Virulence Gene	Biofilm Producer (N=18) n%
SdeAB-B	18 (100.00)	*BsmA*	15 (83.333)
SdeCDE-D	18 (100.00)	*HmsP*	14 (77.77)

SdeXY-Y	15(83.33)	*YmgB*	17(94.44)
SsmE	18(100.00)		

Results of sequencing reactions

Based on the 16S ribosomal nucleic acid sequences in the examined bacterial samples, a thorough phylogenetic tree was produced. This phylogenetic tree was included in our screened bacterial sample together with the other deposited DNA sequences, linked with their closely related sequences in a neighbor-power-joining manner. This complex's tree required the presence of a single distinct species, which corresponds to the tree's only included nucleic acid sequence. Serratia marcescens was this species. The BLAST program placed this sample close to the Gene Bank (Camacho et al., 2009). The results of the sequence alignment revealed that these isolates are *S. marcescens.* The sequences were deposited in the BLAST website's gene bank under the accession numbers (OR342160.1, OR342161.1, OR342158.11, OR342157.1, OR342156.1, OR342163.1, OR342155.1, OR342162.1, OR342164.1).

The comprehensive phylogenetic analysis was based on the partial sequence of the 16S rRNA gene in local *Serratia marcescens* isolates that were used for genetic confirmative detection analysis. The evolutionary distances were computed using the Maximum composite-like hood method by the UPGMA phylogenetic tree (MEGA 10 version). The local *Serratia marcescens* isolates were shown to be closely related to NCBI-Blast *Serratia marcescens.*

Table 5. NCBI-BLAST Homology sequence identity of local isolates of *Serratia marcescens* by 16s rRNA gene sequencing ID in the gene bank and nucleotide sequence identity from the NCBI

Local isolates		Country isolates		
Name	Accession No.	No.Country	Accession No.	Identity
S.marcescens No. 1	OR342155.1	USA	OP986817.1	99%
S.marcescens No. 2	OR342156.1	China	FJ479790.1	99 %
S.marcescens No. 3	OR342157.1	Saudi Arabia	AB571066.1	99 %
S.marcescens No. 4	OR342158.1	India	MT263018.1	99 %
S.marcescens No.5	OR342159.1	USA	NR036886.1	97
S.marcescens No. 6	OR342160.1	Nigeria	MN960115.1	98

Mutations in Sequences of Efflux Pump Genes

Eighteen *Serratia marcescens* isolates were selected, and nucleotide sequences of target genes were detected using a sequencer in South Korea (Macrogen Company). Thereafter, the Geneious program version 10 was used to identify mutations in target genes. Later, the mutations detected in the sequence of these efflux pump genes were compared with those detected in *S. marcescens*in other countries provided in the NCBI. After that, these mutations in bacterial efflux pump genes were recorded at NCBI.

Discussion

A major concern in the field of human medicine is the increasing prevalence of multidrug resistance, specifically the emergence of resistance to extended-spectrum beta-lactamase among *Serratia*species. As a result, numerous studies have been conducted to investigate the epidemiology of these resistance enzymes. In a recent study, it was found that the highest resistance observed among all tested isolates was towards amoxicillin, cefalothin, cefotaxime, and piperacillin. On the other hand, the isolates exhibited higher sensitivity to imipenem, ciprofloxacin, and meropenem (Harada, Charles, Poeppelmeier, and Rondinelli, 2019)In a similar research study conducted by Zaric et al. in 2023, they also examined the resistance patterns of *Serratia* species. However, further details about their specific findings were not provided. In the past, third-generation cephalosporins, fluoroquinolones, and aminoglycosides were commonly used to treat *S. marcescens* infections. However, certain clinical isolates of *Serratia marcescens* have now developed multidrug resistance to these medications (Merkier et al., 2013). This resistance is primarily due to the emergence of extended-spectrumbeta-lactamases (ESBLs), which are the result of mutations in traditional plasmid-encoded β-lactamases. These mutations broaden the enzymatic activity, allowing them to hydrolyze a wider range of broad-spectrum agents such as cefotaxime, ceftazidime, and cefepime (Lynch et al., 2013).The findings of this study align with the results reported by Roya et al. (2017), indicating that *Serratia marcescens* remains largely susceptible to antibiotics. However, there is an increasing prevalence of multiple antimicrobial resistance among human clinical isolates, particularly to critically important antibiotics et al., 2017). Multiple antibiotic resistances have implications for treatment policies in bacterial infections and are indicative of heightened virulence. S. marcescens naturally exhibits resistance to a wide range of antimicrobial agents, known as multidrug resistance. This multidrug resistance is often associated with the presence of multidrug efflux pumps in S. marcescens (Anfal et al., 2011).

The results of the current study did agree with the findings of the researcher (Suresh et al., 2016). In India, it was shown that about 15% of gram-negative bacteria had multiple resistance to antibiotics, and this percentage was due to the possession of bacteria pump efflux, as it reached the results positive for the phenotypic detection of flow pumps (27.90%), as for the results reached by (Helmy and Kashef, 2017). which showed that bacterial isolates have different types of positive bacterial isolation for flow pump phenotype detection (40.54%).This study shows *Serratia marcescens'* ability to phenotypically detect biofilm formation by two methods: Congo red agar and microtiter plate. It was found that 18 (100%) were biofilm formations. while the results, including genotype detection biofilm genes, BsmA gene at the percentage of 15 (83.3%), *Hmsp*gene at the percentage of 14 (77.7%), and *YmgB* gene at the percentage of 17 (94.4%). These results are consistent with studies by Ray *et al.* (2017) and Ramanathan *et al.* (2018), who also investigated the phenotype and genotype of biofilm formation in *Serratia marcescens* isolated from clinical samples. Despite the extensive understanding of *Serratia marcescens'* ability to adhere to various substrates, the specific mechanism by which it attaches to both biotic and abiotic surfaces remains unknown (Koo and Yamada, 2016). Biofilm production is one of the virulence factors of

S. marcescens using Congo red agar, it was found that 18 (90%) were producing biofilm while 2 (10%) only were not producing biofilm. However, at molecular detection targeting the biofilm gene, all positive 20 (100%) isolates were confirmed to have this gene by conventional PCR assay.(Hamza and Al-Hassani, 2023). Molecular detection targeting the efflux pump genes showed all positives at 18 (100%), so it was the result (*SdeAB-B* gene at the percentage 18 (100%), *SdeCDE-D* gene at the percentage 18 (100%), *SdeXY-Y*gene at the percentage 15(83.3%), and *SsmE*gene at the percentage 18 (100%)). Isolates were confirmed to have these genes by conventional PCR assays. Finally, theresults revealed that S. marcescens isolates have 16SrRNA genes at 100 %. Using a DNA sequencer technique to determine the sequence of nucleotides. The results revealed the similarity of the genes in local isolates. *Serratia marcescens* is a known opportunistic pathogen that can cause infections and develop antibiotic resistance. In this study, the aim was to investigate the presence of *S. marcescens* in various clinical samples, such as urinary tract infections, wound infections, and respiratory infections. 18 isolates were obtained from samples containing this pathogen, which corresponds to previous studies (Aggarwal et al., 2017; Christine et al., 2019). Mutations in bacteria can be caused by various factors, including the indiscriminate use of antibacterial agents, prolonged exposure to antibiotics, or high concentrations of antibiotics that stimulate the development of resistance (Dalvi and Worobec, 2012; Martino et al., 2018). These mutations can affect gene expression and protein synthesis, leading to changes in bacteria's susceptibility to antibiotics. In the case of S. marcescens, mutations in the genes of the efflux pump can lead to changes in transport and ejection functions, affecting the resistance of bacteria to antibiotics. The S. marcescens flow pump system is an important resistance mechanism that acts against different groups of antibiotics (Kim et al., 2015). It consists of genes such as *SdeAB-B, SdeCDE-D, SdeXY-Y*, and *SsmE,* which encode transport proteins responsible for regulating antibiotic transport and expulsion out of the bacteria. The presence of efflux pump genes in a high percentage of *S. marcescens* isolates causing nosocomial infections has been reported in previous studies (Ferreira et al., 2020). Studies have shown that inactivating or deleting efflux pump genes can reduce antibiotic resistance in S. marcescens, while transferring these genes into the bacteria can increase multidrug resistance (Hornsey *et al.,* 2010; Thomas *et al*., 2015; Sandner-Miranda *et al.,* 2018). The efflux pump system in *S. marcescens* is characterized by important genes such as *SdeB, SdeD, SdeY,* and *SsmE*. These genes are located on plasmids within the bacteria, allowing them to persist in challenging environments. They encode transport proteins that play a role in regulating the internal environment of the bacteria and facilitating the removal of antibiotics and their toxic substances (Sandner-Miranda et al., 2018). Consequently, bacterial resistance to antibiotics can be acquired, intrinsic, or transient, leading to increased difficulties in treating infections with different classes of antibiotics (Zingg *et al.,* 2017; Thomas *et al*., 2015; Omololu-Aso *et al*., 2021).A study investigating S. marcescens isolates from hospitals found that these bacteria are responsible for various nosocomial infections, and the genes *SdeB, SdeY,* and *HasF*associated with efflux pumps were detected in 88.9% of all isolates Ferreira, R. L.,et al .,2020).The other study found new mutations in efflux pump genes *SdeB, SdeY,* and *HasF* in the

genome of S. marcescens isolated from Iraqi patients(Hamzah and Saleem, 2023). Therefore understanding the mutations and mechanisms involved in efflux pump genes is crucial for developing effective strategies to combat antibiotic resistance in *S. marcescens*. Overall, this study provides valuable insights.Into the prevalence of *S. marcescens* and its antibiotic resistance mechanisms, particularly related to efflux pump genes.

Conclusion

*Serratia marcescens*is one of the important causes of nosocomial infections and has been an important pathogen responsible for many infections, especially urinary tract infections (UTIs). All isolates of *Serratia marcescens*bacteria isolated from different clinical infections showed multiple resistance to antibiotics, with the highest resistance to the antibiotic beingAmoxicillin + Clavulanic acid AMC) (91.59%). The results of the current study showed that all isolates of *Serratia marcescens* bacteria can produce biofilm. Serratia marcescensbacterial isolates showed positive results for phenotypic detection of flow pumps at a rate of 14 (77.7%). The results of the current study showed that most Serratia marcescensbacterial isolates possess SdeAB-B, SdeCDE-D, SdeXY-Y, and SsmE flow pump genes that encode protein efflux pumps by 18 (100%), 18 (100%), 15 (83.3%) and 18 (100%). The results of the current study showed that most Serratia marcescensBacterial isolates possess the biofilm genes HmsP, BsmA, and YmgA in a ratio of 14 (100%),15(83.3%) and 17(94.4%). Multiple new mutations have been recorded and all of these isolates are registered with NCBI. Furthermore, our findings indicate this pump is overexpressed in S. marcescensmultidrug-resistant strains

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