Original Article

Evaluation of Mutations in Efflux Pump Genes in *Serratia Marcescens*

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Abstract

This study aimed to evaluate any mutation in efflux pump genes (*SdeB*, *SdeY*, *HasF*) in *Serratia marcescens* (*S. marcescens*) isolated from UTI, wound and sputum samples and compared them with global genes in NCBI. These genes play an important role in resistance against different types of antibiotics by a mechanism called efflux pump. Identification of bacteria was done according to morphological and biochemical tests and VITEK-2 compact system. 24% of all samples contained this bacterium. After DNA extraction, efflux pump genes were amplified by using specific primers. All isolates of *S. marcescens* were found to have these genes. Sequencing results of these genes depicted mutations which were different from global sequences in NCBI. Later, sequences of these genes were documented in NCBI under accession numbers: *Serratia marcescens* ASH-2 *SdeB* GenBank: LC647797.1, *Serratia marcescens* ASH-4 *SdeY* GenBank: LC647799.1 and *Serratia marcescens* ASH-1 *Hasf* GenBank: LC647796.1.

Keywords: Efflux pump genes, SdeB gene, SdeY gene, HasF gene, Mutation.

Introduction

S. marcescens is a gram negative bacteria first diagnosed by an Italian pharmacist in 1918. The bacterium is bacillus shaped, motile, non-endospore forming, unable to produce oxidase and grows in a difficult environment because of its ability to produce DNase enzyme. Moreover, it appears as a red pigment on culture media because of producing prodigiosin. In addition, it is able to produce toxins, DNase, lipase, gelatinase, hemolysin, proteases, chitinase, chloroperoxidase, alkaline phosphatase, prodigiosin and enzymes responsible for increased resistance against new generation of antibiotics like betalactamse, extended spectrum betalactamase and metalo betalactamase.

S. marcescens is a facultative anaerobe that causes illnesses in human body like infection in many organs and tissues including urinary tract infection, respiratory tract infection, skin infection and blood stream infection.[1-3] Increase in the rate of infection with these bacteria is due to multidrug resistance against different groups of antibiotics. This resistance occurs through different mechanisms including efflux pump, modification in pores of outer membrane, change in target sites of antibiotics binding, modification of metabolic pathways, production of betalactamase, extended spectrum betalactamase and metalo betalactamase.[4-6]

Efflux pump mechanism helps bacteria in removing antibiotic from inside to outside of the cell which reduces accumulation of drug and increases resistance against antibiotics. Gene expression of efflux pump system happens through different mechanisms but the major one is nodulation-cell division (RND)-type efflux pump.[7-9] Genome of *S. marcescens* contains resistance genes including SdeAB, SdeCDE and SdeXY which encode different types of RND-type efflux pumps that play role in multidrug resistance against antibiotics.[10,11] This study evaluates mutation in nucleotide sequence of efflux pump genes i.e. *SdeB* gene, *SdeY* gene and *HasF* gene in multidrug resistance *S. marcescens*.

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Materials and Methods

Isolation and Identification of S. marcescens

A total of 50 samples each from urine, sputum and wound infection were obtained from different patients in major hospitals in Baghdad. All these samples were cultured on blood and MacConkey agar for 18-24 h at 37 °C. Then, primary diagnosis was carried out by examining the appearance and morphology of colony and routine biochemical tests.[12] Final diagnosis of *S. marcescens* was based on VITEK-2 Compact system and target genes i.e. *SdeB* gene, *SdeY* gene and *HasF* gene.

Extraction of DNA from S. marcescens and Amplification of Target Genes

The DNA was extracted from *S. marcescens* by specific promega® kit. Then these genes were amplified by using suitable oligonucleotides (primers) according to annealing temperatures mentioned in table-1.[13]

Gel Electrophoresis

Agarose gel of 1.5% was used to visualize PCR products of target genes. The 1500bp DNA ladder was used to confirm the relevant product size (Fig.1,2,3). In this gel, 1µl of ethidium bromide (10mg/ml) was used and later was run in 1X TAE buffer.[14]

Table-1: Requirements of amplification process of efflux pump genes

Genes	Primers	Sequences (5′ to 3′)	Annealing Temperature
SdeB	Forward primer	AGATGGCCGATAAGCTGTTG	55.4 °C
	Reverse primer	CAGCGTCCAGCTTTCATACA	
SdeY	Forward primer	TCCATCAACGAAGTGGTGAA	55.5 °C
	Reverse primer	GTTTATCGAGAAGCCGAACG	
HasF	Forward primer	CATGTCGAAATGGCGCCAAC	57.5 °C
	Reverse primer	TTGTAGGCGTTGATGCTGCT	

Mutations in Sequences of Efflux Pump Genes

Fifteen isolates of *S. marcescens* isolates were selected and nucleotide sequences of target genes were detected by using sequencer in south Korea (Macrogen company). Thereafter, Geneious program version -9 was used to identify mutations in target genes. Later, the mutations detected in sequence of these efflux pump genes were compared with that detected in *S. marcescens* in other countries provided in NCBI. After that, these mutations in bacterial efflux pump genes were recorded in NCBI.

Results

Identification of S. marcescens

Out of 150 samples, a total of 36 isolates (24%) of *S. marcescens* were detected in blood and MacConkey culture.[12] These results were certified by VITEK-2 Compact system and sequences of genes *SdeB*, *SdeY* and *HasF*. Among these 36 isolates, 14 were from burn infection, 10 from UTI and 12 from sputum.

Amplification of SdeB gene, SdeY gene and HasF

The amplified products of efflux pump genes were run on agarose gel electrophoresis and were found in all isolates of *S. marcescens*. The product sizes of *SdeB, SdeY* and *HasF* genes were about 170 bp, 190 bp, and 800 bp respectively (Figures 1,2,3) .

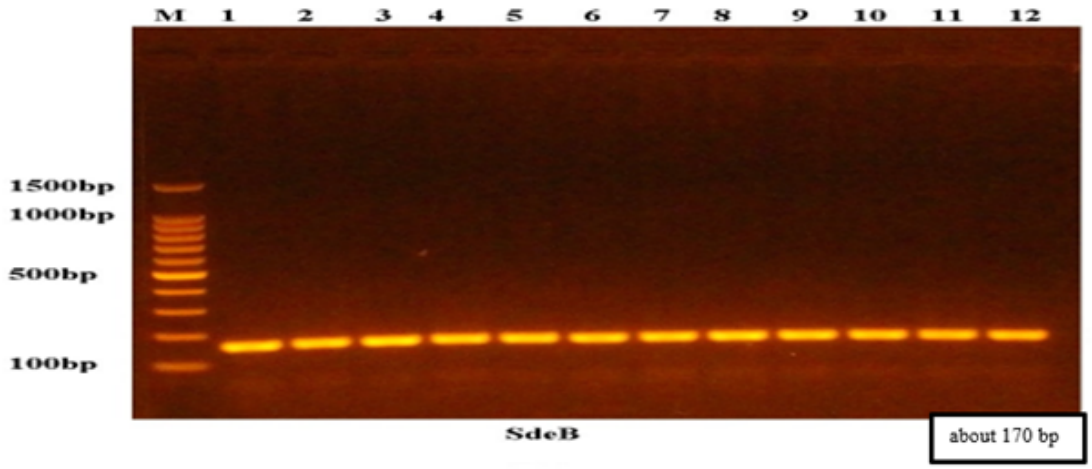

Figure 1-Amplified products of SdeB (about 170 bp) efflux pump gene in S. marcescens isolates shown on agarose gel. M=DNA ladder.

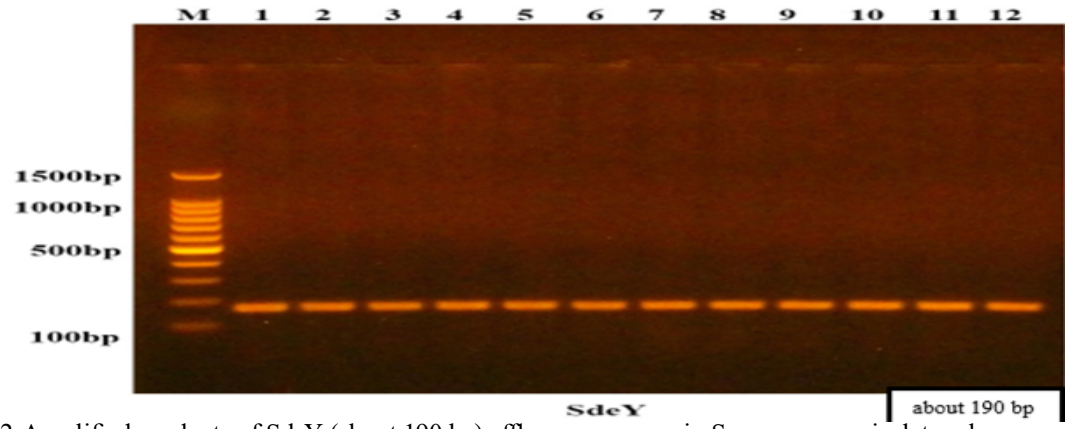

Figure 2-Amplified products of SdeY (about 190 bp) efflux pump gene in S. marcescens isolates shown on agarose gel. M=DNA ladder.

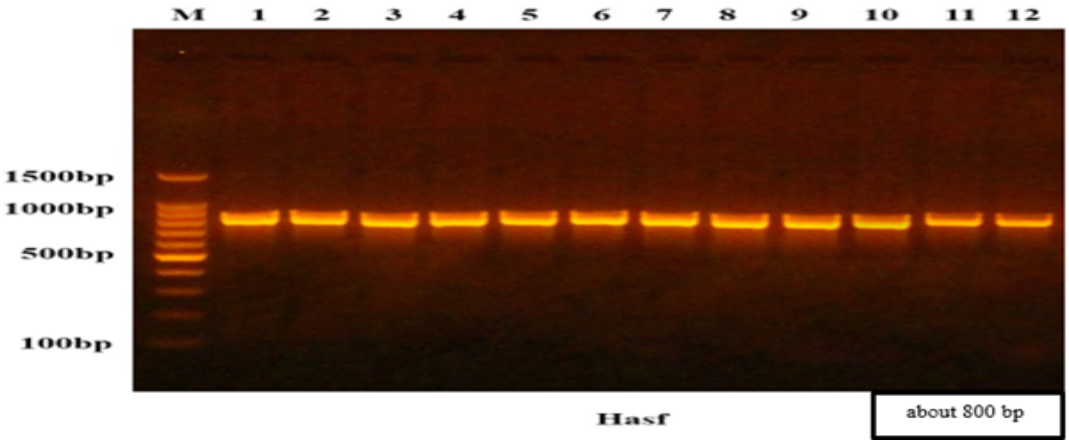

Figure 3-Amplified products of Hasf (about 800 bp) efflux pump gene in S. marcescens isolates shown on agarose gel. M=DNA ladder.

Mutation in Efflux Pump Genes

The sequencing results of efflux pump genes were evaluated for mutations by using Geneious program version -9 and by aligning the sequences with those of same bacteria in NCBI Different mutations were found in in coding region of these efflux pump genes (Figures 4,5,6).

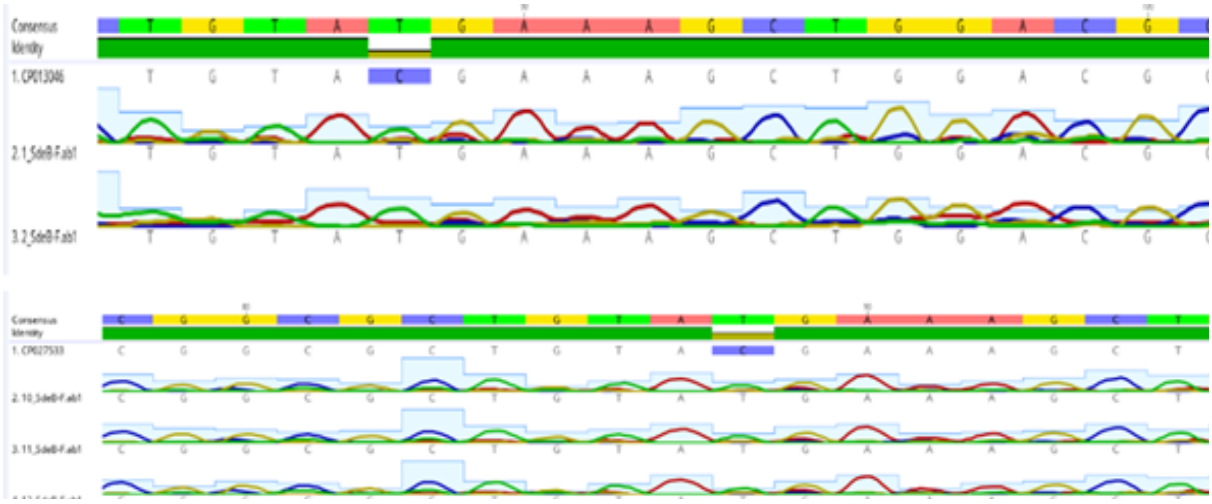

Figure 4-Mutation in sequence of *SdeB* efflux pump gene in *S. marcescens* compared with global (CP027533 , CP013046) in NCBI.

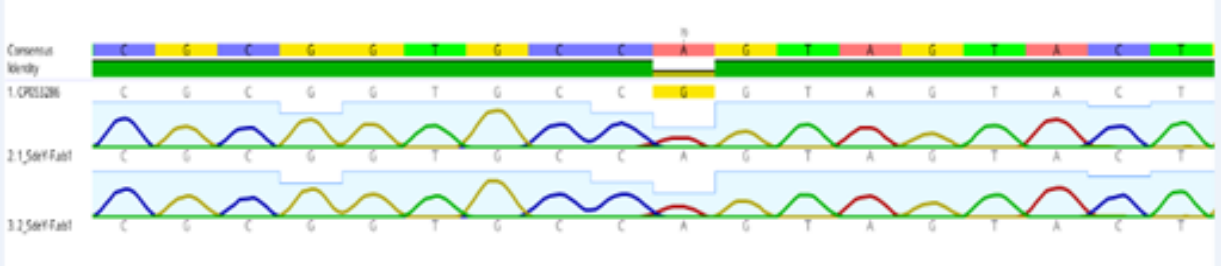

Figure 5-Mutation in sequence of SdeY efflux pump gene in S. marcescens compared with global (CP053286) in NCBI.

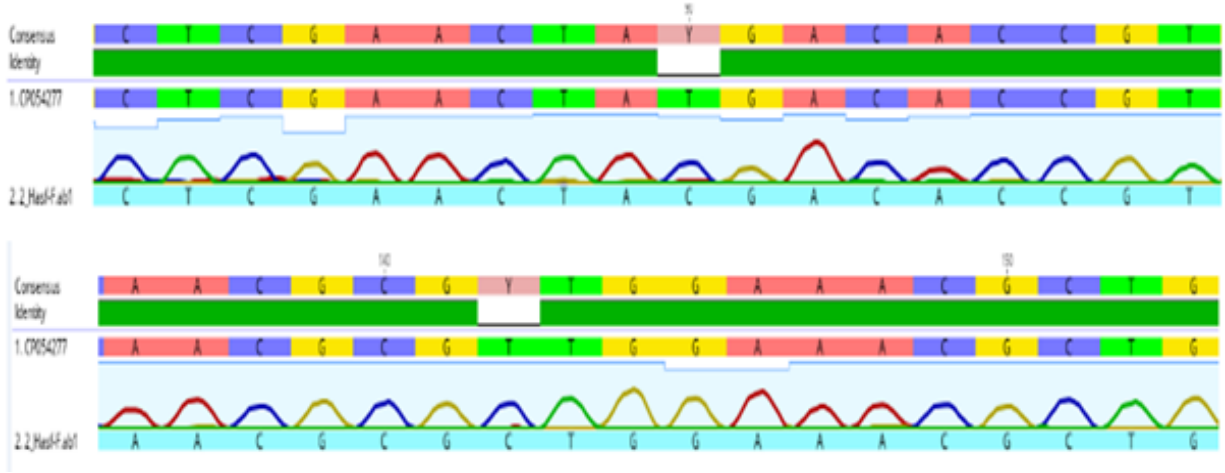

Figure 6-Mutation in sequence of Hasf efflux pump gene in S. marcescens compared with global (CP054277) in NCBI.

Registration of Mutations from Iraqi S. marcescens in NCBI

After comparing with the sequence of same bacteria registered in NCBI, the sequence of efflux pump genes were registered in NCBI. This was due to the mutations found in sequences of these genes in *S. marcescens* isolated from patients in Iraq. Later, the sequences were accepted in NCBI under accession numbers *Serratia marcescens* ASH-2 *SdeB* GenBank: LC647797.1, *Serratia marcescens* ASH-4 *SdeY* GenBank: LC647799.1 and *Serratia marcescens* ASH-1 *Hasf* GenBank: LC647796.1.

Discussion

S. marcescens is considered an opportunistic pathogen that has different factors which lead to the development of infection and antibiotic resistance.[15] It can be isolated from different samples like urinary tract infection, wound infection and sputum[16,17] and in present study, 24% of all samples contained this pathogen. These bacteria become resistant to drugs through different mechanisms including efflux pump.[18,19] This system has various genes including *SdeB*, *SdeY* and *HasF,* which play role in drug resistance. The present study found all isolates of *S. marcescens* to have these genes. Efflux pump mechanism helps bacteria in removing antibiotics from inside to outside of the bacterial cell which leads to multidrug resistance and continuous infection with *S. marcescens*.[20,21]

Evaluation of sequence of these efflux pump genes showed that *S. marcescens* isolated from patients in Iraq developed mutation in sequence of these genes.[22] These mutations were different from those already documented in NCBI, therefore, they were later registered in NCBI under accession numbers *Serratia marcescens* ASH-2 *SdeB* GenBank: LC647797.1, *erratia marcescens* ASH-4 *SdeY* GenBank: LC647799.1 and *Serratia marcescens* ASH-1 *Hasf* GenBank: LC647796.1.

Mutations in *S. marcescens* may be useful or harmful to bacteria.[23] There are different reasons which cause mutations in bacteria e.g. random use of antibacterial agents for short or long period or high concentration of antibiotics that may stimulate genome of these pathogens to develop resistance by gene expression modification that encodes for new enzymes which converts antibiotic from active to inactive state. This leads to the requirement of another group of antibiotics and bacterial resistance occurs in new form with new enzyme. Also, the mutations in coding region of gene especially start or stop codon may affect the translation of mRNA and gene expression and may encode for inactive protein that makes bacteria more susceptible to antibiotics.[24,25]

Efflux pump system in *S. marcescens* is an important resistance mechanism because this pathway acts against all groups of antibiotics like cephalosporin, penicillin, monobactam, metalobactam, sulphanamide, aminoglycoside

and other groups which enter inside the bacteria and exit from the cell.[26] This efflux pump system has important genes like *SdeB* gene, *SdeY* gene and *HasF* gene. This system is located in plasmid of bacteria therefore it contributes in their persistency in difficult environment. These genes encode for transport proteins which help in regulation inside the bacteria and remove antibiotics and their toxic substance.[6] Therefore, the resistance of bacteria against antibiotics may be acquired, intrinsic or transient that increases difficulty in treatment with different groups of antibiotics.[9,27,28] A study found that *S. marcescens* isolated from hospitals causes different nosocomial infections and SdeB, SdeY and HasF efflux pump genes were found in 88.9% of all isolates.[29] Another study found low effect on antibiotic resistance due to efflux pump genes in mutant bacteria. Local environmental conditions or physical and chemical mutants may stimulate different types of mutations like transversion, insertion or deletion.[30] A study reported *S. marcescens* to become susceptible to antimicrobial agent due to deletion mutation in efflux pump genes.[31] Likewise, a study observed reduction in level of antibiotics resistance in clinical *S. marcescens* when their efflux pump genes were inactivated by suicide plasmids containing the R6Kg origin of replication.[13] Another study found an increase in multi drug resistance in these bacteria because of the presence of multiple efflux pump genes in their genome.[6] Interestingly, a study genetically transferred the efflux pump genes into these bacteria and observed an increase in multidrug resistance in them.[27] The current study found new mutations in efflux pump genes *SdeB*, *SdeY* and *HasF* in genome of *S. marcescens* isolated from Iraqi patients.

Conclusion

Efflux pump genes *SdeB*, *SdeY* and *HasF* code for proteins which increase resistance of *S. marcescens* against different types of antibiotics. This study concluded that all isolates of this bacterium have these genes in its genome and the sequencing results show mutations in these genes which are different from those already documented in NCBI. These mutations may affect the resistance level of these bacteria.

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