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Evaluation of Phenotyping and Genotyping Characterization of *Serratia marcescens* after Biofield Treatment

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Abstract

Serratia marcescens (*S. marcescens*) is Gram-negative bacterium, associated with hospital-acquired infections (HAIs), especially urinary tract and wound infections. The present study was aimed to evaluate the impact of biofield treatment on phenotyping and genotyping characteristics such as antimicrobial susceptibility, biochemical reactions, biotype, DNA polymorphism, and phylogenetic relationship of *S. marcescens* (ATCC 13880). The lyophilized cells of *S. marcescens* were divided into three groups (G1, G2, and G3). Control group (G1) and treated groups (G2 and G3) of *S. marcescens* cells assessed with respect to antimicrobial susceptibility, and biochemical reactions. In addition to that, samples from different groups of *S. marcescens* were evaluated for DNA polymorphism by Random Amplified Polymorphic DNA (RAPD), and 16S rDNA sequencing in order to establish the phylogenetic relationship of *S. marcescens* with different bacterial species. The treated cells of *S. marcescens* showed an alteration of 10.34% and 34.48% antimicrobials in G2 and G3 on 10^{th} day, respectively as compared to control. The significant changes of biochemical reactions were also observed in treated groups of *S. marcescens*. The RAPD data showed an average range of 16-49.2% of polymorphism in treated samples as compared to control. Based on nucleotide homology sequences and phylogenetic analysis, the nearest homolog genus-species was found to be *Pseudomonas fluorescence*. These findings suggest that biofield treatment can prevent the emergence of absolute resistance to the useful antimicrobials against *S. marcescens*.

Keywords: Antimicrobials; Biofield treatment; Polymorphism; Microbial resistance; RAPD; *S. marcescens*

Introduction

Currently, many microorganisms have been acquired the resistance to number of antibiotics and other antimicrobial agents, which were effectively used earlier to cure a microbial infections. The antimicrobial resistant microbes (including bacteria, viruses, fungi, and parasites) can survive in antimicrobial drugs therapy. Therefore, regular treatments are ineffective. The frequent and improper use or misuse of antimicrobial medicines accelerates the emergence of drug-resistant microorganism, which was further spread by meagre infection control and poor sanitary conditions [1]. *Serratia marcescens* (*S. marcescens*) is a rod-shaped Gram-negative bacteria, belongs to family *Enterobacteriaceae*. It is a facultative anaerobic bacterium that can grow in presence and absence of oxygen at temperatures 30°C to 37°C. *S. marcescens* become an opportunist pathogen causing nosocomial infections and commonly involved in hospital-acquired infections (HAIs); specially urinary tract infections (UTIs), pneumonia, septicemia, meningitis and wound infections. Recently, *S. marcescens* drastically acquired the resistance to several existing antimicrobials like penicillin by decreasing the permeability and by β-lactamase to cleave the β-lactam ring of penicillin; fluoroquinolones (nalidixic acid, ciprofloxacin, ofloxacin, and norfloxacin), by proton dependent multidrug resistance (MDR) efflux pumps [2,3]. Therefore, development of effective antimicrobial therapy against *S. marcescens* is very needful for human health. Recently, biofield treatment came in focus that can cure the microbial infection by changing the microbial susceptibility against the antimicrobial drugs.

The relation between mass-energy was described by Friedrich, then after Einstein gave the well-known equation $E=mc^2$ for light and mass [4,5]. The mass (solid matter) is consist of energy and once this energy vibrates at a certain frequency, it gives physical, atomic and structural properties like shape, size, texture, crystal structure, and atomic weight to the matter. Similarly, human body also consists of vibratory energy particles like neutrons, protons, and electrons. Due to the vibration of these particles in the nucleus, an electrical impulse is generated [6]. Consequently, as per Ampere-Maxwell-Law, varying of these electrical impulses with time generates magnetic field, which cumulatively form electromagnetic field [7,8]. Thus, human has the ability to harness the energy from environment or universe and can transmit into any living or nonliving object(s) around the Globe. The objects always receive the energy and responding into useful way that is called biofield energy and the process is known as biofield treatment. Mr. Mahendra Trivedi's biofield treatment (The Trivedi Effect®) has been applied to transform the structural, physical, and chemical properties of materials in several fields like material science [9-16], agriculture [17-19], and biotechnology [20,21]. The biofield treatment has considerably altered the genotype of the microbes and thereby changed in susceptibility to antimicrobials [22-24].

After consideration of clinical significance of *S. marcescens* and significant impact of biofield treatment on microbes, we felt a detailed investigation was required to evaluate the effect of biofield treatment on *S. marcescens*. After that, the organism was assessed in relation to antimicrobials susceptibility and biotyping based on various

biochemical reactions. We also explored the genotyping of this organism using polymerase chain reaction (PCR) based methodologies of randomly amplified polymorphic DNA (RAPD) and 16S rDNA sequencing techniques. To the best of our knowledge, this is the first report that explores the impact of biofield treatment on *S. marcescens*.

Materials and Methods

Two vials of *S. marcescens* [American Type Culture Collection (ATCC) 13880] were procured from MicroBioLogics, Inc., USA, in sealed packs, and stored as per the recommended storage conditions until further use. The anti-microbial susceptibility, biochemical reactions, and biotype number were evaluated on MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA) using Negative Breakpoint Combo 30 (NBPC30). DNA Fingerprinting by RAPD analysis (using Ultrapure Genomic DNA Prep Kit; Cat KT 83) and the 16S rDNA sequencing studies were carried out using Ultrapure Genomic DNA Prep Kit; Cat KT 83 (Bangalore Genei, India). All the tested antimicrobials, biochemicals and other reagents were procured from Sigma-Aldrich.

Study design

The microorganisms were grouped as per study design like bacterial cell were divided in to three groups G1 (control), G2 (treatment, revived), and G3 (treatment, lyophilized). The treatment groups (G1 and G2) were in sealed pack and handed over to Mr. Trivedi for biofield treatment under laboratory condition. Mr. Trivedi provided the treatment through his energy transmission process to the treated groups without touching the samples. After that, G2 group was assessed for antimicrobial susceptibility and biochemical reactions on 5^{th} and 10^{th} day of incubation; and G3 group was assessed on 10^{th} day of treatment. The treated groups were compared with respect to control.

Investigation of antimicrobial susceptibility of *S. marcescens*

Antimicrobial susceptibility of *S. marcescens* was investigated with the help of automated instrument, MicroScan Walk-Away® using Negative Breakpoint Combo 30 (NBPC30) panel as per the manufacturer's instructions [25]. Briefly, after inoculation and rehydration with a standardized suspension of *S. marcescens*, were incubated at 35°C for 16 h. The minimum inhibitory concentration (MIC) and a qualitative susceptibility like susceptible (S), intermediate (I), inducible β-lactamases (IB), and resistant (R) were determined by observing the lowest antimicrobial concentration showing growth inhibition [26]. In the present study, the following 29 antimicrobials were used like amikacin, amoxicillin/k-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, cefotetan, cefoxitin, ceftazidime, cefuroxime, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, nitrofurantoin, norfloxacin, piperacillin, tazobactam, ticarcillin, tobramycin, and vancomycin.

Biochemical studies

The biochemical studies of *S. marcescens* were determined by MicroScan Walk-Away® where, interpretation of biochemical reactions for microbial identification of Gram-negative organisms resulted in high accuracy [27,28]. In this study, the following 31 biochemicals were used like acetamide, adonitol, arabinose, arginine, cetrimide, cephalothin, citrate, colistin, esculin hydrolysis, nitrofurantoin, glucose, hydrogen sulfide, indole, inositol, kanamycin, lysine, malonate, melibiose, nitrate, oxidation, galactosidase, ornithine, oxidase, raffinose, rhamnose, sorbitol, sucrose, tartrate, tobramycin, urea, and Voges-Proskauer.

Biotype number

The biotype number of *S. marcescens* was determined by MicroScan Walk-Away® processed panel data utilizing biochemical reactions data [25].

Random Amplified Polymorphic DNA (RAPD) analysis

Three inoculums (one for control and other two for treatment named as treatment A and B) were prepared of *S. marcescens* samples. Two inoculums (treatment samples A and B) were subjected to Mr. Trivedi's biofield treatment. After that, the treated samples were sub-cultured by taking 1% inoculum and inoculated to fresh 5 mL medium and labeled as treatment A-1 and treatment B-1, respectively. All samples were incubated at 37°C with 160 rpm for 18 h. Subsequently, the cultures were spun down, and genomic DNA was isolated for control and treated samples using Genomic DNA Prep Kit (Bangalore Genei, India). RAPD was performed with all samples of *S. marcescens* using five RAPD primers, which were labelled as RBA8A, RBA13A, RBA20A, RBA10A and RBA15A. The PCR mixture contained 2.5 µL each of buffer, 4.0 mM each of dNTP, 2.5 µM each of primer, 5.0 µL each of genomic DNA, 2 U each of Taq polymerase, 1.5 µL of $MgCl_2$ and 9.5 µL of water in a total of 25 µL with the following PCR amplification protocol; initial denaturation at 94°C for 7 min, followed by 8 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, and extension at 72°C for 2 min; and 35 cycle of denaturation at 94°C for 1 min, annealing at 38°C for 1 min, and extension at 72°C for 1.5 min; and the final extension at 72°C for 7 min. Amplified PCR products from all samples (control and treated) were separated on 1.5 % agarose gels at 75 volts, stained with ethidium bromide and visualized under UV illumination.

Amplification and gene sequencing of 16S rDNA

Genomic DNA was isolated from *S. marcescens* cells by using genomic purification Kit, according to the instructions of manufacturer. 16S rDNA gene (~1.5 kb) was amplified by universal primers; forward primer (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer (3′-ACGGTCATACCTTGTTACGACTT-5′). Amplified products were subjected to electrophoresis in 1.0% agarose gel, stained with ethidium bromide and visualized under UV light in a gel documentation unit (BioRad Laboratories, USA). The PCR amplified fragment was purified from the agarose gel using a DNA Gel Extraction Kit. Sequencing of amplified product was done on commercial basis from Bangalore Genei, India. The 16S rDNA sequences obtained were aligned and compared with the sequences stored in Gene Bank data base available from National Center for Biotechnology Information (NCBI) using the algorithm BLASTn program. Multiple sequence alignment/phylogenetic tree were established using MEGA3.1 molecular software [29].

Results

Assessment of antimicrobial susceptibility

The effect of biofield treatment on *S. marcescens* to susceptibility pattern and MIC of selected antimicrobials are summarized in Tables 1

and 2, respectively. The data were analyzed and compared with respect to control. The treated cells of *S. marcescens* showed an alteration of 10.34% and 34.48% in G2 and G3 group on 10th day, respectively of antimicrobials susceptibility among all tested antimicrobials as compared to control. Studying the effect of biofield treatment in the antibiogram of *S. marcescens*, revealed that the amikacin and tobramycin were converted from resistance to susceptible on 10th day of G3 group as compared to control. Aztreonam, cefotetan, ceftazidime, cefuroxime and chloramphenicol were converted from resistance to intermediate on 10th day of biofield treatment of G3 group as compared to control. The cefepime and cefotaxime were converted from resistance to intermediate on 10th day of G2 treated cells and complete susceptibility was observed for gentamycin and cefepime on 10th day of G3 treated cells as compared to control (Table 1). It was also observed that there was reduced activity of inducible β-lactamase of aztreonam, cefotaxime, cefotetan, ceftazidime, and ceftriaxone antimicrobials. The MIC values of amikacin, aztreonam, cefepime, cefotetan, ceftazidime, gentamicin and tobramycin were decreased about two-folds; whereas about four-folds decrease in MIC values of cefotaxime and ceftriaxone on 10th day of G2 treated cells as compared to control (Table 2).

S. No.	Antimicrobial	Control	G2		G3
		G1	5th day	10th day	10th day
1	Amikacin	R	R	R	S
2	Amoxicillin/K-clavulanate	R	R	R	R
3	Ampicillin/Sulbactam	R	R	R	R
4	Ampicillin	R	R	R	R
5	Aztreonam	R	R	R	IB
6	Cefazolin	R	R	R	R
7	Cefepime	R	R	I	S
8	Cefotaxime	R	R	I	IB
9	Cefotetan	R	R	R	IB
10	Cefoxitin	R	R	R	R
11	Ceftazidime	R	R	R	IB
12	Cefuroxime	R	R	R	R
13	Ceftriaxone	I	I	IB	IB
14	Cephalothin	R	R	R	R
15	Chloramphenicol	R	R	R	I
16	Ciprofloxacin	S	S	S	S
17	Gatifloxacin	S	S	S	S
18	Gentamicin	R	R	R	S
19	Imipenem	S	S	S	S
20	Levofloxacin	S	S	S	S
21	Meropenem	S	S	S	S
22	Moxifloxacin	S	S	S	S
23	Nitrofurantoin	R	R	R	R
24	Norfloxacin	S	S	S	S
25	Piperacillin	IB	IB	IB	IB
26	Tazobactam	IB	IB	IB	IB
27	Ticarcillin	IB	IB	IB	IB
28	Tobramycin	R	R	R	S
29	Vancomycin	S	S	S	S

G stands for group; I: intermediate; S: susceptible; R: resistant; IB: inducible β-lactamase.

Table 1: Effect of biofield treatment on *S. marcescens* to susceptibility pattern of selected antimicrobials.

S. No.	Antimicrobial	Control	G2		G3
		G1	5th day	10th day	10th day
1	Amikacin	>32	>32	>32	≤16
2	Amoxicillin/K-clavulanate	≥16/8	≥16/8	≥16/8	≥16/8
3	Ampicillin/ Sulbactam	≥16/8	≥16/8	≥16/8	≥16/8
4	Ampicillin	≥16	≥16	≥16	≥16
5	Aztreonam	>16	>16	>16	≤8
6	Cefazolin	≥16	≥16	≥16	≥16
7	Cefepime	>16	>16	16	≤8
8	Cefotaxime	>32	>32	32	≤8
9	Cefotetan	>32	>32	>32	≤16
10	Cefoxitin	≥16	≥16	≥16	≥16
11	Ceftazidime	>16	>16	>16	≤8
12	Cefuroxime	>16	>16	>16	>16
13	Ceftriaxone	32	32	≤8	≤8
14	Cephalothin	≥16	≥16	≥16	≥16
15	Chloramphenicol	>16	>16	>16	16
16	Ciprofloxacin	≤1	≤1	≤1	≤1
17	Gatifloxacin	≤2	≤2	≤2	≤2
18	Gentamicin	>8	>8	>8	≤4
19	Imipenem	≤4	≤4	≤4	≤4
20	Levofloxacin	≤2	≤2	≤2	≤2
21	Meropenem	≤4	≤4	≤4	≤4
22	Moxifloxacin	≤2	≤2	≤2	≤2
23	Nitrofurantoin	≥64	≥64	≥64	≥64

24	Norfloxacin	≤4	≤4	≤4	≤4
25	Piperacillin	≤16	≤16	≤16	≤16
26	Tazobactam	≤16	≤16	≤16	≤16
27	Ticarcillin	≤16	≤16	≤16	≤16
28	Tobramycin	>8	>8	>8	≤4
29	Vancomycin	≤2	≤2	≤2	≤2
G stands for group; MIC data are presented in µg/mL.					

Table 2: Effect of biofield treatment on *S. marcescens* to MIC of selected antimicrobials.

Organism identification by biochemical reactions

The biochemical reactions of *S. marcescens* are presented in Table 3. In the present study, acetamide, cetrimide, indole, inositol, and oxidase biochemical reactions of control and treated cells of *S. marcescens* showed negative biochemical reactions.

S. No.	Code	Biochemical	Control	G2		G3
			G1	5th day	10th day	10th day
1	ACE	Acetamide	-	-	-	-
2	ADO	Adonitol	+	+	+	+
3	ARA	Arabinose	+	+	+	-
4	ARG	Arginine	+	+	-	-
5	CET	Cetrimide	-	-	-	-
6	CF8	Cephalothin	+	+	+	+
7	CIT	Citrate	+	+	+	+
8	CL4	Colistin	+	+	+	+
9	ESC	Esculin hydrolysis	+	+	+	+
10	FD64	Nitrofurantoin	+	+	+	+
11	GLU	Glucose	+	+	+	+
12	H2S	Hydrogen sulfide	+	+	+	-
13	IND	Indole	-	-	-	-
14	INO	Inositol	-	-	-	-
15	K4	Kanamycin	+	+	+	-
16	LYS	Lysine	+	+	+	+
17	MAL	Malonate	+	+	+	-
18	MEL	Melibiose	+	+	+	-
19	NIT	Nitrate	+	+	+	+
20	OF/G	Oxidation	+	+	+	+
21	ONPG	Galactosidase	+	+	+	+
22	ORN	Ornithine	+	+	+	+
23	OXI	Oxidase	-	-	-	-
24	RAF	Raffinose	+	+	+	-
25	RHA	Rhamnose	+	+	+	-
26	SOR	Sorbitol	+	+	+	+
27	SUC	Sucrose	+	+	+	+
28	TAR	Tartrate	-	+	+	-
29	TO4	Tobramycin	+	+	+	-
30	URE	Urea	+	+	+	-
31	VP	Voges-Proskauer	+	+	+	+
G stands for group; - (negative); + (positive).						

Table 3: Effect of biofield treatment on *S. marcescens* to biochemical reactions.

Twenty-four of thirty-one biochemical reactions were showed positive reaction for control and two treatment groups. Arginine reaction of treated G2 cells on 10th day was negative and tartrate reaction was positive for the treatment G2 cells on both 5th and 10th day as compared to control. Ten out of thirty one biochemical reactions (32.25 %) of treated cells in G3 were converted from positive to negative reaction, and tartrate biochemical reaction was remain unchanged as negative as compared to control (Table 3).

Organism identification by biotype number

The biotype number of *S. marcescens* was determined by MicroScan Walk-Away® processed panel, using biochemical reactions data. There was no change in biotype number observed in treated G2 cells on 5th day of incubation. However, the significant changes in the biotype number of *S. marcescens* were observed in G2 and G3 on 10th day of incubation as compared to control (Table 4).

Feature	Control	G2		G3
	G1	5th day	10th day	10th day
Biotype Number	7736 7376	7736 7376	7736 5376	7020 5356
Organism Identification Name	*S. marcescens*	*S. marcescens*	*S. marcescens*	*S. marcescens*
G stands for group.				

Table 4: Effect of biofield treatment on *S. marcescens* to biotype number.

Random Amplified Polymorphic DNA (RAPD) analysis

The DNA polymorphic photograph is shown in Figure 1, and the polymorphic bands are marked by arrows.

The percentage of polymorphism was calculated using following equation:

Percent polymorphism=A/B×100;

Where, A=number of polymorphic bands in treated sample; and B=number of polymorphic bands in control.

The results of DNA polymorphic patterns are shown in Tables 5 and 6. The level of polymorphism was found about an average range of 16-49.2% of polymorphism in treated samples as compared to control in *S. marcescens*.

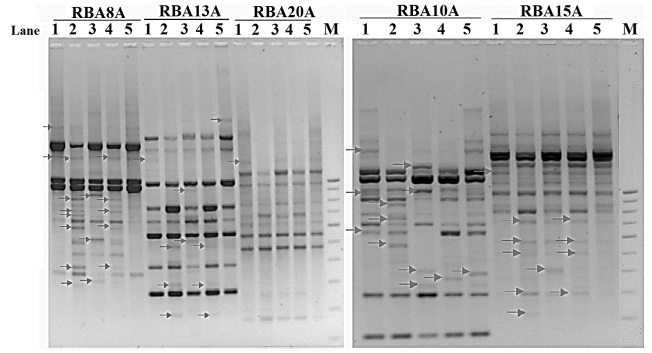

Figure 1: Random amplified polymorphic-DNA fragment patterns of *S. marcescens* generated using five RAPD primers, RBA 8A, RBA 13A, RBA 20A, RBA 10A and RBA 15A. 1, Control; 2, Treated A; 3, Treated A-1; 4, Treated B; 5, Treated B-1; M: 100 bp DNA Ladder.

S. No.	Primer	Nucleotide sequence (5'-3')	Band scores	Common bands in control and treated	Unique band				
					Control	TSA	TSA-1	TSB	TSB-1
1	RBA 8A	GTTTC GCTCC	17	5	2	4	3	3	0
2	RBA 13A	GTGGA TCCGA	14	8	1	2	1	1	1
3	RBA 20A	GCGAT CCCCA	8	7	1	0	0	0	0
4	RBA 10A	CCGCA GCCAA	17	5	1	3	3	2	3
5	RBA 15A	AAGAG CCCGT	15	9	1	2	1	2	0

TSA: treated sample A; TSA-1: treated sample A-1; TSB: treated sample B; TSB-1: treated sample B-1.

Table 5: DNA polymorphism analyzed by random amplified polymorphic DNA (RAPD) analysis.

Primer	C and TSA	C and TSA-1	C and TSB	C and TSB-1	TSA and TSA-1	TSB and TSB-1	TSA and TSB	TSA-1 and TSB-1
RBA 8A	90%	50%	70%	20%	66%	38%	20%	30%
RBA 13A	40%	30%	40%	20%	45%	40%	0.0%	10%
RBA 20A	10%	0.0%	10%	0.0%	41%	10%	0.0%	0.0%
RBA 10A	46%	53%	30%	30%	58%	44%	16%	23%
RBA 15A	60%	20%	50%	10%	50%	28%	10%	10%
Average polymorphism	49.2 %	30.6%	40%	16%	52%	32%	9.2%	14.6%

C: control; TSA: treated sample A; TSA-1: treated sample A-1; TSB: treated sample B; TSB-1: treated sample B-1

Table 6: Level of polymorphism between control and treated samples.

16S rDNA genotyping

The 16S rDNA sequence was determined in *S. marcescens*. The alignment and comparison of the gene sequences were performed with the sequences stored in Gene Bank database available from NCBI using the algorithm BLASTn program. The nearest homolog genus-species of *S. marcescens* was found to be *P. fluorescens* (Accession No. DQ439976). Some other close homologs of *S. marcescens* were can be found from the alignment as shown in Table 7.

Alignment view	ID	Alignment result	Sequence description
	8A	0.96	Sample studied
	EU233275	0.96	*Serratia marcescens* strain RJT
	AB061685	0.98	*Serratia marcescens*
	EF208030	0.97	*Serratia marcescens* strain A3
	EF194094	0.97	*Serratia marcescens* strain H3010
	DQ439976	0.98	*Pseudomonas fluorescens* strain ost5
	AB091837	0.98	*Pseudomonas fluorescens*
	EU036987	0.97	*Serratia nematodiphila* strain DZ0503SBS1
	EF627046	0.97	*Serratia marcescens* strain cocoon-1
	AJ233431	1	*Serratia marcescens* (strain DSM 30121)
	DQ417332	0.96	*Serratia marcescens* strain 6CW

Table 7: The closest sequences of *S. marcescens* from sequence alignment using NCBI GenBank and ribosomal database project (RDP).

The distance matrix based on nucleotide sequence homology data are presented in Figure 2. Based on nucleotides homology and phylogenetic analysis the microbe (Sample 8A) was detected to be *S. marcescens* (GenBank Accession Number: EU233275). Phylogenetic

tree was established using BLAST-Webpage (NCBI). According to Figure 2, ten different related bacterial species and *S. marcescens* were selected as Operational Taxonomic Units (OTUs) in order to investigate the phylogenetic relationship of *S. marcescens* among other ten other bacterial species. There were 1506 base nucleotides of 16S rDNA gene sequences were analyzed and multiple alignment were constructed using ClustalW in MEGA3.1. The numbers of base substitutions per site from pairwise distance analysis between sequences are shown in Table 7. All results are based on the pairwise analysis of 11 sequences. According to the data in Figure 2, the lowest value of genetic distance from *S. marcescens* was 0.000 base substitutions per site. All pairwise distance analysis was carried out using the p-distance method in MEGA3.1. The proportion of remarked distance, sometimes also called p-distance and showed as the number of nucleotide distances site. Values in Table 7 were programmed into Figure 2 with optimal bootstrap consensus tree. In the phylogram, there were eleven OTUs. Based on the phylogenetic tree and 16S rDNA sequencing, the nearest homolog genus-species of *S. marcescens* was found to be *P. fluorescens* (Figure 3).

		1	2	3	4	5	6	7	8	9	10	11
EF208030	1	---	0.993	0.993	0.992	0.999	0.991	0.9992	0.997	0.997	0.993	0.992
AB091837	2	0.007	---	0.999	0.999	0.993	0.997	0.996	0.993	0.991	1.000	0.996
AB061685	3	0.007	0.001	---	0.999	0.993	0.997	0.996	0.992	0.991	0.999	0.996
EU036987	4	0.008	0.001	0.001	---	0.993	0.999	0.996	0.992	0.99	0.999	0.996
EF194094	5	0.001	0.007	0.007	0.007	---	0.992	0.992	0.997	0.997	0.993	0.992
EF627046	6	0.009	0.003	0.003	0.001	0.008	---	0.995	0.991	0.988	0.997	0.995
EU233275	7	0.008	0.004	0.004	0.004	0.008	0.005	---	0.992	0.990	0.0996	1.000
AJ233431	8	0.003	0.007	0.008	0.008	0.003	0.009	0.008	---	0.995	0.993	0.992
DQ417332	9	0.003	0.009	0.009	0.010	0.003	0.012	0.010	0.005	---	0.991	0.990
DQ439976	10	0.007	0.000	0.001	0.001	0.007	0.003	0.004	0.007	0.009	---	0.996
8A	11	0.008	0.004	0.004	0.004	0.008	0.005	0.000	0.008	0.010	0.004	---

Figure 2: Distance matrix based on nucleotide sequence homology. All results are based on the pair wise analysis of 11 sequences. Analysis was conducted using the p-distance method in MEGA3.1.

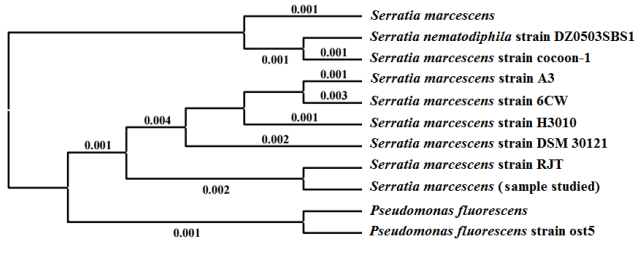

Figure 3: Phylogenetic relationship between *S. marcescens* and other bacteria in same genera based on 16S rDNA sequences.

Discussion

The increasing incidence of antimicrobial resistance is getting more global attention. Antibiotic multi-resistant Gram-negative bacteria pose a risk to public health [30]. Extended spectrum β-lactam antibiotics have been extensively used for treatment of severe Gram-negative infections since four decades. Although, bacterial resistance has emerged rapidly due to the production of ESBLs [31]. *Enterobacteriaceae* producing ESBL are emerging as a threatening cause of both hospital and community acquired infection, as they are often resistant to standard antimicrobial choices [32,33]. It is generally thought that patients infected by an ESBL-producing organism are at an increased risk of treatment failure [34]. Recently, an increasing percentage of ESBL-producing *S. marcescens* has been detected worldwide with a significant impact on the clinical course of disease. ESBL are enzymes produced by some *S. marcescens* species that inactivate many antimicrobials such as penicillins, expanded spectrum cephalosporins, monobactams including older β-lactam antimicrobial agents and are inhibited by clavulanic acids, imipenem, sulbactam or monobactam [35,36].

In the present work, we investigated the impact of biofield treatment on *S. marcescens* and evaluated the antimicrobials susceptibility pattern, biochemical reactions, biotype number, and DNA polymorphism of this microbe. The treated cells of *S. marcescens* showed an alteration in susceptibility of 10.34% and 34.48% antimicrobials of G2 and G3 group on 10^{th} day, respectively, as compared to control (Table 1). A significant change was found for a few antimicrobials to their antimicrobial susceptibility from resistant to intermediate and resistant to susceptible at 10^{th} day of treated G2 and G3 group, respectively (Table 1). MIC values of about 10.34% and 34.48% antimicrobials were decreased in G2 and G3 group, respectively on 10^{th} day (Table 2). Studying the effect of biofield treatment in the antibiogram of *S. marcescens* revealed that the amikacin converted from resistance to susceptible on 10^{th} day of G2 cells as compared to control. The MIC values of some antimicrobials such as cefotetan, ceftazidime, gentamicin, and tobramycin were decreased by about two-folds in treated G2 on 10^{th} day and four-folds decrease were observed for cefotaxime and ceftriaxone at 10^{th} day of treated G3 (Table 2). The *S. marcescens* also showed the substantial changes in biochemical reactions pattern towards a few biochemicals on 10^{th} day of treated G3 group as mentioned in Table 3. The alterations in biochemical reactions pattern were further supported by the determination of biotype number of *S. marcescens*, which was changed from 7736 7376 (control) to 7736 5376 and 7020 5356 for treated G2 and G3 on 10^{th} day, respectively (Table 4). DNA fingerprinting by RAPD analysis using five primers was carried out on control and treated samples. DNA profiles were compared within and across control and treated groups. The RAPD data showed an average range of 16-49.2% of polymorphism in treated samples as compared to control, indicated polymorphism occurred in treated groups. The highest change in DNA sequence was observed in treated groups with RBA 8A primer as compared to control; a negligible change was found in treated group with RBA 20A primer as compared to control. BLASTn analysis revealed studied sample (8A) gene sequence shared 99% identity to the sequence of *S. marcescens*. The phylogenetic tree diagram predicted the closest species of *S. marcescens* was found to be as *P. fluorescens* (Figure 3). Based on these results, it is expected that biofield treatment has the scope to be a cost effective and alternative approach than the existing antimicrobial therapy in near future.

Conclusion

The results suggest that there has an impact of biofield treatment on antimicrobial susceptibility, biochemical reactions, and DNA polymorphism of *S. marcescens*. These changes were found in the organism may be due to alteration happened at the genetic and/or

enzymatic level after biofield treatment. Therefore, biofield treatment could be applied to improve the sensitivity of antimicrobials against microbial resistance.

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