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Evaluation of Phenotyping and Genotyping Characteristic of *Shigella sonnei* after Biofield Treatment

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Abstract

Shigella sonnei (*S. sonnei*) is a non-motile, rod shape, clinically significant, Gram-negative bacterium. It is commonly associated with dysentery (shigellosis). Recently, resistance to third and fourth generation cephalosporins and fluoroquinolones has been reported in *S. sonnei*. In the present study, we assessed the effect of biofield treatment on phenotyping and genotyping characteristic of *S. sonnei* (ATCC 9290). The lyophilized samples of *S. sonnei* were divided in three groups (G): G-I (control, revived), G-II (treatment, revived), and G-III (treatment, lyophilized). All these groups (control and biofield treated) were analyzed against antimicrobial susceptibility, biochemical reactions, and biotype number. The 16S rDNA sequencing was carried out to establish the phylogenetic relationship of *S. sonnei* with different bacterial species. The treated cells of *S. sonnei* exhibited an alteration of 3.33%, 10%, and 23.33% of total 30 tested antimicrobials in susceptibility assay for G-II on day 5 and 10 and G-III on day 10, respectively as compared to control. The treated cells of *S. sonnei* showed a significant change of about 12.12%, 12.12%, and 57.58% biochemical reactions out of 33 tests in treated groups of G-II on day 5 and 10 and G-III on day 10, respectively. The biotype number was also changed in treated samples of *S. sonnei*. Based on nucleotide homology sequences and phylogenetic analysis, the nearest homolog species of *S. sonnei* (GenBank Accession Number: EU009190) was identified as *Shigella flexneri* (EF643608). These results revealed that biofield treatment can prevent the absolute resistance in microbe against the existing antimicrobials.

Keywords: Antimicrobial susceptibility; Biofield treatment; 16S rDNA gene sequencing; *Shigella sonnei*

Abbreviations: MIC: Minimum Inhibitory Concentration; ATCC: American Type Culture Collection; NBPC30: Negative Breakpoint Combo 30; NCBI: National Center for Biotechnology Information; WHO: World Health Organization; 16S rDNA: 16Svedberg Unit Ribosomal Deoxyribonucleic Acid; BLAST: Basic Local Alignment Search Tool; Outs: Operational Taxonomic Units

Introduction

Development of antimicrobial resistance in several microbes like bacteria, viruses, fungi, or in parasites has been reported globally in the recent few decades. Frequent and improper use of antimicrobial further accelerated the incidence of microbial resistance [1]. *Shigella sonnei* (*S. sonnei*) is a rod shape, non-motile, facultative anaerobic Gram-negative and lactose-fermenting bacterium. *S. sonnei* is associated with gastrointestinal tract (GIT) infection disease shigellosis in both developed and developing countries, where the sanitation is insufficient [2,3]. *S. sonnei* is usually transmitted by fecal-oral route, direct interpersonal contact, contaminated food, water, or uncooked food. *Shigella* infection is the third most common gastroenteritis after Salmonella and Campylobacter infection in the USA. Recently, *S. sonnei* has become the most prevalent species in the developed world. It is estimated to cause 80–165 million cases of disease and 600,000 deaths annually, worldwide [4]. The *S. sonnei* has been acquired resistant to commonly used antimicrobials like streptomycin, tetracycline, sulfonamide, trimethoprim, and ampicillin. Emergence of extended-spectrum β-lactamases (ESBLs) in *S. sonnei* was also detected in Korea [2,5]. Therefore the multidrug therapy required to treat the infection cause by resistant strain of microbes. However, multiple drug therapy shows serious toxicity and associated adverse effects like neurotoxicity and nephrotoxicity [6]. Due to associated side effects and failure of drug therapy, an alternate treatment approach is required. Recently, an alternate treatment known as biofield energy is reported that inhibits the growth of bacterial cultures [7]. Biofield is an electromagnetic field that permeates and surrounds living organisms and referred as biologically produced electromagnetic and subtle energy field that provides regulatory and communication functions within the human organism [8]. Various internal physiological processes such as blood flow, brain and heart function *etc.* generates biofield. Researchers have attempted different biologic studies and effects of biofield on various biomolecules such as proteins, antibiotics [9], conformational change in DNA [10] etc. 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 [11]. Mr. Mahendra Trivedi's biofield treatment (The Trivedi Effect®) has renowned to alter the various physicochemical characteristics of metals and ceramics [11-17]. Quality and quantity of several agriculture products have been improved by several folds in the biofield treated plants [18-20] and growth and adaptation of the plant were also enhanced with the help of biofield treatment [21,22]. In addition, the biofield treatment has considerably altered the phenotype and biotype of the microbe and subsequently, the susceptibility to antimicrobials was also changed [23-25].

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Based on the knowledge of existing literatures and considering the clinical significance of *S. sonnei*, we evaluated to see the impact of biofield treatment on antimicrobial susceptibility, biochemical reactions pattern, biotype number, and 16S rDNA gene sequencing of the microbe.

Materials and Methods

Two lyophilized vials of *S. sonnei* [American Type Culture Collection (ATCC) 9290] were purchased from MicroBioLogics, Inc., USA. The microbial sample vials were stored as per the suggested storage conditions till further use. The antimicrobial susceptibility study, biochemical reactions pattern, and biotype number were evaluated by MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA) through Negative Breakpoint Combo 30 (NBPC30) panel. The 16S rDNA sequencing was performed using Ultrapure Genomic DNA Prep Kit (Cat KT 83, Bangalore Genei, India).

Biofield treatment

The lyophilized strain of *S. sonnei* were divided into three groups (G) like G-I (control), G-II (treatment, revived), and G-III (treatment, lyophilized). G-I consider as control. No treatment was given. The treatment groups (II and III) 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. Subsequently, group G-I and G-II were assessed on day 5 and 10; and G-III was assessed on day 10. After that, all groups were evaluated for an antimicrobial susceptibility, biochemical reactions pattern, and biotype number [25]. The 16S rDNA gene sequencing of *S. sonnei* was also carried out.

Investigation of antimicrobial susceptibility of *S. sonnei*

The antimicrobial susceptibility of *S. sonnei* was evaluated with the help of automated instrument, MicroScan Walk-Away® using Negative Breakpoint Combo 30 (NBPC30) panel as per the manufactures instructions [26]. The minimum inhibitory concentration (MIC) and a qualitative susceptibility like resistant (R), intermediate (I), and susceptible (S) were determined by analyzing the lowest antimicrobial concentration showing microbial growth inhibition [25]. The antimicrobial sensitivity study was carried out using following 30 antimicrobials such as amikacin, amoxicillin/K-clavulanate acid, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, cefotetan, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, gatifloxacin , gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, nitrofurantoin, norfloxacin, piperacillin, tazobactam, tetracycline, ticarcillin, tobramycin, and trimethoprim/sulfamethoxazole. All these antimicrobials were procured from Sigma-Aldrich.

Biochemical studies

The biochemical reactions of *S. sonnei* were carried out using MicroScan Walk-Away® system where, interpretation of biochemical reactions for microbial identification of Gram-negative organisms resulted in high accuracy [27,28]. The biochemical reactions patterns of control and treated samples of *S. sonnei* were performed using the following 33 biochemicals such as acetamide, adonitol, arabinose, arginine, cetrimide, cephalothin, citrate, colistin, esculin hydrolysis, nitrofurantoin, glucose, hydrogen sulfide, indole, inositol, kanamycin, lysine, malonate, melibiose, nitrate, oxidation-fermentation, galactosidase, ornithine, oxidase, penicillin, raffinose, rhamnose, sorbitol, sucrose, tartrate, tryptophan deaminase, tobramycin, urea, and Voges-Proskauer. All these biochemicals were procured from Sigma-Aldrich.

Biotype number

The biotype number of *S. sonnei* was found out utilizing the MicroScan Walk-Away® processed panel data report, using biochemical reactions data [26].

16S rDNA gene sequencing

Genomic DNA was prepared from biofield treated *S. sonnei* cells using genomic purification Kit, as per the manufacturer's instructions. Subsequently, the 16S rDNA gene (~1.5 kb) was amplified using universal forward primer 5'-AGAGTTTGATCCTGGC-3' and universal reverse primer 5'-GGTTACCTTGTTACGACTT-3'. Subsequently, the amplified products were resolved by gel electrophoresis on 1.0% agarose gel, stained with ethidium bromide, and then visualized under UV light in a gel documentation unit (BioRad Laboratories, USA). The PCR amplified fragment was purified from the agarose gel utilizing a DNA Gel Extraction Kit. The amplified product was sequenced on commercial basis from Bangalore Genei, India. The received 16S rDNA sequences data were aligned and compared with the sequences stored in Gene Bank database of National Center for Biotechnology Information (NCBI) using the algorithm BLASTn program. Finally, the multiple sequence alignment/phylogenetic tree was constructed with help of MEGA 3.1 software utilizing neighbor joining method [29,30].

Results

Antimicrobial susceptibility assay

The antimicrobial sensitivity data were reported in Table 1 and 2. The result showed that the biofield treated *S. sonnei* exhibited a significant alteration in susceptibility assay of about 3.33% (G-II on day 5), 10% (G-II on day 10), and 23.33% (G-III on day 10) of total tested antimicrobials. The antimicrobials ampicillin, aztreonam, cefotaxime, ceftazidime, chloramphenicol, and tetracycline were converted from R → S; simultaneously more than 2 folds decreases in MIC values in lyophilized treated group G-III; cefotaxime showed a decrease in susceptibility from R → I in G-II on day 10. Besides, amoxicillin/K-clavulanate and ampicillin/sulbactam were converted from S → R in G-II and cefepime converted from I → S in G-III on day 10.

Identification of *S. sonnei* by biochemical reactions

The results of biochemical reactions of *S. sonnei* are presented in Table 3, which represent a significant alteration in biochemical reactions of about 12.12% (G-II on day 5 and 10), and 57.58% (G-III on day 10) of total tested biochemicals as compared to control. The biochemicals such as adonitol, cephalothin, citrate, colistin, esculin hydrolysis, hydrogen sulfide, kanamycin, lysine, malonate, melibiose, raffinose, sorbitol, sucrose, tobramycin, urea, and Voges-Proskauer were changed from positive (control) → negative reactions (treated) in G-III microbes. Additionally, arginine was converted from positive to negative reaction in entire treated groups. Nitrofurantoin was converted from positive to negative in G-II on day 5 and G-III on day 10 (Table 3). Tartrate was converted from positive to negative reaction in both G-II and G-III on day 10; and inositol and tryptophan deaminase were converted from negative to positive reaction in G-II on both days (Table 3). All the data were compared as compared to control.

S. No.	Antimicrobial	Control	Treated		
		G-I	G-II		G-III
			Day-5	Day-10	Day-10
1	Amikacin	R	R	R	R
2	Amoxicillin/k- clavulanate	S	S	R	S
3	Ampicillin/sulbactam	S	I	R	S
4	Ampicillin	R	R	R	S
5	Aztreonam	R	R	R	S
6	Cefazolin	I	I	I	I
7	Cefepime	I	I	I	S
8	Cefotaxime	R	R	I	S
9	Cefotetan	R	R	R	R
10	Cefoxitin	R	R	R	R
11	Ceftazidime	R	R	R	S
12	Ceftriaxone	S	S	S	S
13	Cefuroxime	R	R	R	R
14	Cephalothin	R	R	R	R
15	Chloramphenicol	R	R	R	S
16	Ciprofloxacin	S	S	S	S
17	Gatifloxacin	S	S	S	S
18	Gentamicin	I	I	I	I
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	S	S	S	S
26	Piperacillin/tazobactam	S	S	S	S
27	Tetracycline	R	R	R	S
28	Ticarcillin/k-clavulanate	S	S	S	S
29	Tobramycin	R	R	R	R
30	Trimethoprim/ sulfamethoxazole	S	S	S	S

Table 1: Effect of biofield treatment on *Shigella sonnei* to antimicrobial susceptibility pattern of selected antimicrobials
G, stands for group; The control group G-I and G-II were accessed on day 5 and 10; and G-III was accessed on day 10, after the biofield treatment; I, intermediate; S, susceptible; R, resistant.

Effect of biofield treatment on biotype number

Biotype number of *S. sonnie* was determined on MicroScan Walk-Away® processed panel. The result was demonstrated an alteration in biotype number of *S. sonnie* in the entire treated groups G-II and G-III (Table 4). However, the species (*S. sonnei*) was remained unchanged in the entire treated group.

16S rDNA gene sequencing

The 16S rDNA sequence was determined in *S. sonnei* and shown in Figure 1. The alignment and comparison of the gene sequences were performed with the sequences stored in Gene Bank data base available from NCBI using the algorithm BLASTn program. As evidenced from nucleotides homology and phylogenetic analysis the sample 6A (*S. sonnei*) was identified as the same species (*S. sonnei*) with 99% identity of gene sequencing data. Ten bacterial species and *S. sonnei* were considered as Operational Taxonomic Units (OTUs) to facilitate the investigation of phylogenetic relationship of *S. sonnei* among other related species. Total 1500 base nucleotide of 16S rDNA gene sequences were compared by multiple alignments with the help of ClustalW in MEGA3.1 [30], and the data are shown in Table 5.

S. No.	Antimicrobial	Control	Treated		
		G-I	G-II		G-III
			Day-5	Day-10	Day-10
1	Amikacin	>32	>32	>32	>32
2	Amoxicillin/k- clavulanate	≤8/4	≤8/4	>16/8	≤8/4
3	Ampicillin/sulbactam	≤8/4	16/8	>16/8	≤8/4
4	Ampicillin	>16	>16	>16	≤8
5	Aztreonam	>16	>16	>16	≤8
6	Cefazolin	16	16	16	16
7	Cefepime	16	16	16	≤8
8	Cefotaxime	>32	>32	32	≤16
9	Cefotetan	>32	>32	>32	>32
10	Cefoxitin	>16	>16	>16	>16
11	Ceftazidime	>16	>16	>16	≤8
12	Ceftriaxone	≤8	≤8	≤8	≤8
13	Cefuroxime	>16	>16	>16	>16
14	Cephalothin	16	16	16	16
15	Chloramphenicol	>16	>16	>16	≤8
16	Ciprofloxacin	≤1	≤1	≤1	≤1
17	Gatifloxacin	≤2	≤2	≤2	≤2
18	Gentamicin	>8	>8	>8	>8
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	Piperacillin/tazobactam	≤16	≤16	≤16	≤16
27	Tetracycline	>8	>8	>8	≤4
28	Ticarcillin/k-clavulanate	≤16	≤16	≤16	≤16
29	Tobramycin	>8	>8	>8	>8
30	Trimethoprim/ sulfamethoxazole	≤2/38	≤2/38	≤2/38	≤2/38

G, stands for group; MIC data is presented in µg/mL

Table 2: Effect of biofield treatment on *Shigella sonnei* to minimum inhibitory concentration (MIC) of selected antimicrobials.

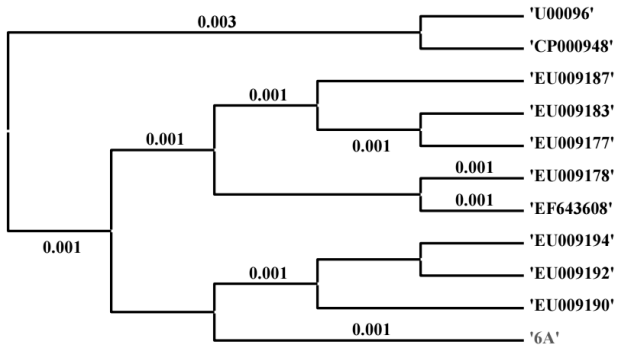

Figure 1: Phylogenetic tree of the partial 16S rDNA gene sequencing using MEGA 3.1 software by Neighbor joining method.

As evidenced from Table 6, the lowest value of genetic distance from *S. sonnei* was 0.002 base substitutions per site. The nearest homolog genus-species of *S. sonnei* (Genbank accession number: EU009190) was determined by analyzing the 16S rDNA sequencing and phylogenetic tree, and found to be *Shigella flexneri* (Genbank

S. No.	Code	Biochemical	Control	Treated		
			G-I	G-II		G-III
				Day-5	Day-10	Day-10
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-fermentation/ glucose	+	+	+	+
21	ONPG	Galactosidase	+	+	+	+
22	ORN	Ornithine	+	+	+	+
23	OXI	Oxidase	-	-	-	-
24	P4	Penicillin	+	+	+	+
25	RAF	Raffinose	+	+	+	-
26	RHA	Rhamnose	+	+	+	+
27	SOR	Sorbitol	+	+	+	-
28	SUC	Sucrose	+	+	+	-
29	TAR	Tartrate	+	+	-	-
30	TDA	Tryptophan deaminase	-	+	+	-
31	TO4	Tobramycin	+	+	+	-
32	URE	Urea	+	+	+	-
33	VP	Voges-Proskauer	+	+	+	-

G, stands for group; -, (negative); +, (positive)

Table 3: Effect of biofield treatment on *Shigella sonnie* to biochemicals reactions pattern.

Feature	Control	Treated		
	G-I	G-II		G-III
		Day-5	Day-10	Day-10
Biotype	7736 7376	7776 5776	7776 5776	4300 1010
Organism Identification Name	*S. sonnei*	*S. sonnei*	*S. sonnei*	*S. sonnei*

Table 4: Effect of biofield treatment on *Shigella sonnei* to alteration in biotype.

accession number: EF643608). The distance matrix was prepared based on nucleotide sequence homology data and presented in Table 6. All pairwise distance analysis was carried out employing the p-distance method in MEGA3.1 software.

Discussion

Antimicrobial resistance is a major global threat to public health, reported by World Health Organization (WHO). WHO also reported a post-antibiotic era, where people will die from simple microbial infections that have been curable for decades. Microbes naturally mutate and ultimately become immune to antimicrobials. Unfortunately, due to misuse of antimicrobials like over-prescribing by doctors or improper uses by patients is causing it to happen in much faster than expected. Similarly, *S. sonnei* has been acquired resistant to commonly used antimicrobials like tetracycline, streptomycin, trimethoprim, sulfonamide, and ampicillin. Further, emergence of extended-spectrum β-lactamases (ESBLs) was also detected in *S. sonnei* in some Asian countries like Korea [2,3,31].

Alignment view	AN	Alignment results	Sequence description
	6A	0.99	Sample studied
	CP000948	0.99	*Escherichia coli* strain DH10B,
	U00096	0.99	*Escherichia coli* strain K12 sub str. MG1655
	EU009194	0.99	*Shigella sonnei* strain FBD020
	EU009192	0.99	*Shigella sonnei* strain FBD018
	EU009190	0.99	*Shigella sonnei* strain FBD016
	EU009187	0.99	*Shigella flexneri* strain FBD002
	EU009178	0.99	*Shigella boydii* strain FBD007
	EF643608	0.99	*Shigella flexneri* strain FBD001shig
	EU009183	0.99	*Shigella dysenteriae* strain FBD012
	EU009177	0.99	*Shigella boydii* strain FBD006

AN: Accession Number

Table 5: The closest sequences of *Shigella sonnei* from sequence alignment using NCBI GenBank and Ribosomal database project (RDP).

Due to increasing the number of clinical specimens, cost-effectiveness, and convenient interfaces with hospital information systems and laboratory the uses of automated or semi-automated systems for the susceptibility testing and identification of microbes has been increased recently [32]. Therefore, we also utilized the MicroScan Walk-Away® system for analysis of antimicrobial sensitivity, biochemical reactions, and biotyping. The overall result of antimicrobial susceptibility of biofield treated *S. sonnei* suggested that biofield treatment has significantly alerted the sensitivity of microbes in both side (either S → R or R → S) as compared to control. The biochemical reactions of treated cells of *S. sonnei* were altered in the range of 12.11 to 57.58% in treated group as compared to control, which could be due to some alteration happened in metabolic enzyme systems and/or genetic system. It was also found that there was an alteration of biotype number in treated groups of *S. sonnei*. Based on the BLASTn analysis, the sample 6A was identified as *S. sonnei*. The closest homologues species of *S. sonnei* was identified as *Shigella flexneri*. The present study revealed that biofield treatment can alter the sensitivity of antimicrobials against *S. sonnei*. It seems that biofield treatment can play a potential role to circumvent the severe microbial infection in the fast and cost effective way as compared to modern medication.

Conclusion

Altogether data suggest that there was an impact of biofield treatment on antimicrobial susceptibility, biochemical reactions pattern, and biotype number of *S. sonnei*. To the best of our knowledge, this is the first report describing the significant impact of biofield treatment on *S. sonnei* in relation to change the sensitivity of antimicrobials.

AN		1	2	3	4	5	6	7	8	9	10	11
EU009194	1	—	0.998	0.995	0.998	1.000	0.998	0.995	0.997	0.997	1.000	0.998
EU009187	2	0.002	—	0.994	0.999	0.998	0.999	0.994	0.999	0.999	0.998	0.997
U00096	3	0.005	0.006	—	0.994	0.995	0.994	1.000	0.993	0.993	0.995	0.994
EU009178	4	0.002	0.001	0.006	—	0.998	0.999	0.994	0.998	0.998	0.998	0.997
EU009192	5	0.000	0.002	0.005	0.002	—	0.998	0.995	0.997	0.997	1.000	0.998
EF643608	6	0.002	0.001	0.006	0.001	0.002	—	0.994	0.998	0.998	0.998	0.997
CP000948	7	0.005	0.006	0.000	0.006	0.005	0.006	—	0.993	0.993	0.995	0.994
EU009183	8	0.003	0.001	0.007	0.002	0.003	0.002	0.007	—	1.000	0.997	0.997
EU009177	9	0.003	0.001	0.007	0.002	0.003	0.002	0.007	0.000	—	0.997	0.997
EU009190	10	0.000	0.002	0.005	0.002	0.000	0.002	0.005	0.003	0.003	—	0.998
6A	11	0.002	0.003	0.006	0.003	0.002	0.003	0.006	0.003	0.003	0.002	—

AN: Accession Number

Table 6: Distance matrix based on nucleotide sequence homology (Using Kimura-2 Parameter).

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