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Antibiogram Pattern of *Shigella flexneri*: Effect of BioField Treatment

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Abstract

Shigellosis is a major public health burden in India and its neighboring countries due to infection of *Shigella* species. The current study was attempted to investigate the effect of biofield treatment on *Shigella flexneri* (*S. flexneri*) with respect of antimicrobial susceptibility assay, biochemical characteristics and biotyping. The American Type Culture Collection (ATCC 9199) strain of *S. flexneri* was used in this experiment. The study was conducted in revived and lyophilized state of *S. flexneri*. Both revived (Group; Gr. II) and lyophilized (Gr. III) strain of *S. flexneri* were subjected to Mr. Trivedi's biofield treatment. Gr. II was assessed on day 5 and day 10, while Gr. III on day 10 after biofield treatment with respect to control (Gr. I). The antimicrobial susceptibility of *S. flexneri* showed 35% alteration in Gr. II on day 10 while no alteration were observed on day 5 (Gr. II) and in Gr. III as compared to control. The minimum inhibitory concentration (MIC) values of biofield treated *S. flexneri* also showed significant (46.88%) alteration in Gr. II on day 10 while no alteration were observed on day 5 (Gr. II) and in Gr. III as compared to control. It was observed that overall 24.24% biochemical reactions were altered in which 21.21% alteration was found in Gr. II on day 10 with respect to control. Moreover, biotype number was changed in Gr. II on day 10 with identification of new organism *i.e. Edwardsiella tarda* (40015042) as compared to untreated strain of *Shigella* species (40010000). The result suggested that biofield treatment has significant impact on *S. flexneri* in revived treated cells (Gr. II) on day 10 with respect to antimicrobial susceptibility, MIC, biochemical reactions pattern and biotyping.

Keywords: *Shigella flexneri;* Biofield treatment; Antimicrobial susceptibility; Minimum inhibitory concentration; Biochemical reaction; Biotype; Shigellosis

Abbreviations: MIC: Minimum Inhibitory Concentration; ATCC: American Type Culture Collection; NBPC 30: Negative Breakpoint Combo 30; MSM: Men Who have Sex with Men; NICED: National Institute of Cholera and Enteric Diseases; CDC: Centers for Disease Control and Prevention

Introduction

Shigellosis (*i.e.* bacillary dysentery) is a major public health burden in developing countries. Increased incidence of antibiotic resistance in *Shigella flexneri* (*S. flexneri*), constitute a major public health concern. *S. flexneri* is a non-motile, non-spore forming, non-lactose fermenting, Gram-negative, facultative anaerobic and rod shape bacterium, belongs to *Enterobacteriaceae* family. It mainly causes infection through contaminated food/water or with fecal matter [1,2]. *Shigella* spp. causes acute gastrointestinal infections by invasion to the mucosa (colonic epithelium), where it releases potent cytotoxin (shigatoxin) that causes severe local mucosal inflammation or ulceration due to low pH tolerant to acidic environment [3,4]. It does not produce gas from carbohydrates but ferment glucose predominately which is one of the characteristic features [5]. Manifestation of clinical complications in *S. flexneri* infected patients such as shigellosis (watery diarrhoea with mild vomiting), reactive arthritis and hemolytic uremic syndrome [6]. Shigellosis is predominantly a sexually transmitted disease, caused by *Shigella* spp. with direct oral-anal contact conferring the highest risk in HIV infected host [7]. Since, 2009 there has been an increase in UK-acquired infections amongst men who have sex with men (MSM). An outburst of *S. flexneri* amongst MSM has also described in North America in 2007 [8].

According to the United States Centers for Disease Control and Prevention (CDC) reports, that more than one million deaths of the developing world occur per year due to infections with *Shigella* spp. [1]. From literature it has been also reported that *Shigella* species infect 450,000 persons annually in the United States [7], resulting in 6200 hospitalizations and 70 deaths [9]. National Institute of Cholera and Enteric Diseases (NICED) reported that high frequency of resistance in *Shigella* against many of the first line antimicrobial agents (multidrug resistant) have been reported in recent years [10]. Fluoroquinolone or ceftriaxone are the drug of choice to treat shigellosis. However, due to high tendency of multidrug resistance globally including fluoroquinolones and newer cephalosporins, particularly in South and East Asia [11], some alternative strategies are needed to treat against strain of *S. flexneri*.

Harold Saxton Burr, had performed the detailed studies on correlation of electric current with physiological process and concluded that every single process in the human body had an electrical significance [12]. Furthermore, the energy exists in various forms and there are several ways to transfer the energy from one place to another such as electromagnetic waves, electrochemical, electrical and thermal etc. Similarly, the human nervous system consists of neurons, which have the ability to transmit information and energy in the form of electrical signals [13]. According to Rivera-Ruiz et al. electrocardiography has been extensively used to measure the biofield of human body [14]. Thus, human has the ability to harness the energy from environment or Universe and can transmit into any living or nonliving object(s) around

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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. Trivedi's unique biofield treatment (The Trivedi Effect®) has been known to transform the structural, physical and thermal properties of several metals and ceramic in materials science [15-17], improved the overall productivity of crops [18,19], altered characteristics features of microbes [20-22] and improved growth and anatomical characteristics of various medicinal plants [23,24].

Due to the clinical significance of this organism and literature reports on biofield treatment as an alternative approach, the present work was undertaken to evaluate the impact of biofield treatment on *S. flexneri* in relation to antimicrobials susceptibility, minimum inhibitory concentration (MIC) and biotyping based on various biochemical characters.

Materials and Methods

S. flexneri, American Type Culture Collection (ATCC 9199) strains were procured from MicroBioLogics, Inc., USA, in two sets A and B. Two different sealed packs were stored with proper storage conditions until further use. The antimicrobial susceptibility, MIC values, biochemical reactions and biotype number were estimated with the help of MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA, USA) using negative breakpoint combo 30 (NBPC 30) panel. All the tested antimicrobials and biochemicals were procured from Sigma-Aldrich (MA, USA).

Experimental design

Two ATCC samples A (revived) and B (lyophilized) of *S. flexneri* were grouped (Gr.). The revived sample A was divided into two parts Gr.I (control) and Gr.II (revived; treatment); likewise, ATCC B was labeled as Gr.III (lyophilized; treatment).

Biofield treatment strategy

The Gr. I remained as untreated. The treatment Gr. II and III in sealed packs were handed over to Mr. Trivedi for biofield treatment under laboratory condition. Mr. Trivedi provided the treatment through his energy transmission process which includes bioenergy emission to the treated groups (Gr. II and Gr. III) without touching the samples. After treatment, sample was handed over in the same condition and stored at standard conditions as per the standard experimental protocol. An optimum precautionary measure were taken while evaluating the study parameters throughout the experiments. The differences in parameters before and after the treatment were noted and compared. Gr.II was assessed at two time point *i.e.* on day 5 and day 10, while Gr. III was assessed on day 10 for antimicrobial susceptibility, MIC, biochemical reactions pattern, and biotyping.

Antimicrobial susceptibility test

Investigation of antimicrobial susceptibility of *S. flexneri* was carried out with the help of automated instrument, MicroScan Walk-Away® using NBPC 30 panel as per the clinical and laboratory standards institute (CLSI) guidelines. The test was carried out on MicroScan which was miniaturized of the broth dilution susceptibility test that has been dehydrated. Briefly, the standardized suspension of *S. flexneri* was inoculated, rehydrated, and then subjected to incubation for 16 hours at 35°C. The detailed experimental procedures and conditions were followed as per the manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, R: Resistant; and I: Intermediate) and MIC were determined by observing the lowest antimicrobial concentration showing inhibition of growth [25].

Biochemical reaction studies

Biochemical reactions of *S. flexneri* were determined using MicroScan Walk-Away®, system with NBPC 30 panel [25].

dentification of organism by biotype number

The biotype number of *S. flexneri* was determined on MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data [25].

Results and Discussion

Antimicrobial susceptibility test

The outcomes of *S. flexneri* susceptibility pattern and MIC values of tested antimicrobials after biofield treatment are summarized in Tables 1 and 2, respectively. The data were analyzed and compared with respect to the control. Antimicrobial susceptibility was carried out in twenty antimicrobials. The revived treated cells (Gr. II) of *S. flexneri* showed a significant alteration in antimicrobial sensitivity pattern *i.e.* 35% (seven out of twenty) on day 10, while did not show any alteration of antimicrobial sensitivity pattern on day 5 (Gr. II) and in Gr. III as compared to the control. Antimicrobials such as amoxicillin/k-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, chloramphenicol, and trimethoprim/sulfamethoxazole showed an alteration of susceptibility pattern from S to R in Gr. II on day 10 as compared to the control. Alteration of resistance pattern of these antimicrobials may be due to the changes at genetic level that could be govern due to biofield energy treatment on *S. flexneri* [26]. According to routine antibiogram studies the resistance responses of chloramphenicol and trimethoprim/sulfamethoxazole and susceptible responses of ciprofloxacillin and imipenem were well supported by literature data [27]. However, these antimicrobials did not show any change on day 5 in Gr. II and on day 10 in Gr. III as compared to the control. Antibiotic *i.e.* ceftazidime showed an alteration of sensitivity pattern from susceptible to intermediate in Gr. II on day 10, while remain unchanged on day 5 (Gr. II) and in Gr. III on day 10 as compared to control. Rest of antimicrobials *i.e.* 65% out of twenty viz. cefepime, cefotaxime, ceftriaxone, ciprofloxacin, gatifloxacin, imipenem, levofloxacin, meropenem, moxifloxacin, piperacillin/tazobactam, piperacillin, tetracycline, and ticarcillin/k-clavulanate did not show any change of antimicrobial sensitivity after biofield treatment with respect to control sample (Table 1). The sensitivity patterns of both cefotaxime and ceftriaxone in control *S. flexneri* sample were matched with literature data [28]. The MIC values of amoxicillin/k-clavulanate and ampicillin/sulbactam were increased from ≤8/4 to >16/8 µg/mL (i.e. two-fold increase) in Gr. II on day 10 while remain unchanged on day 5 (Gr. II) and in Gr. III on day 10 as compared to control. Alteration of MIC values of ampicillin, aztreonam, cefazolin, cefoxitin, cephalothin and chloramphenicol were altered from ≤8 to >16 µg/mL (i.e. two-fold) in Gr. II on day 10 while remain unchanged on day 5 in Gr. II and on day 10 in Gr. III as compared to control. Moreover, change in MIC value of cefotetan from ≤16 to >32 µg/mL (i.e. two-fold) was observed in Gr. II on day 10 after biofield treatment, while did not change on day 5 in Gr. II and on day 10 in Gr. III as compared to control. The MIC value of ceftazidime was changed from ≤8 to 16 µg/mL (i.e. two-fold) on day 10 in Gr. II, while remain unaltered on day 5 in Gr. II and on day 10 in Gr. III as compared to control. The MIC value of cefuroxime was changed from ≤4 to >16 in Gr. II on day 10 as compared to control. The MIC values of extended spectrum β-lactamases (ESBL) a Scrn and b Scrn were changed from ≤4 to >4 and ≤1 to >1 respectively in Gr. II on day 10 while no change of MIC values were observed in Gr. II (on day 5)

and Gr. III as compared to control. Antimicrobials *i.e.* nitrofurantoin and trimethoprim/sulfamethoxazole showed an alteration of MIC values from ≤32 to >64 µg/mL (i.e. two-fold) and ≤2/38 to >2/38 µg/mL respectively in Gr. II on day 10 while remain unchanged on day 5 (Gr. II) and in Gr. III as compared to control. Overall, 46.88% (fifteen out of thirty two) antimicrobials showed altered MIC values after biofield treatment in Gr. II on day 10 while MIC values were remained unchanged on day 5 in Gr. II and on day 10 in Gr. III as compared to control. Seventeen, out of thirty two (53.13%) tested antimicrobials *viz.* amikacin, cefepime, cefotaxime, ceftriaxone, ciprofloxacin, gatifloxacin, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, norfloxacin, piperacillin/tazobactam, piperacillin, tetracycline, ticarcillin/k-clavulanate and tobramycin did not show any alteration in MIC value in treated cells of *S. flexneri* as compared to control (Table 2).

Biochemical reactions studies

Study of biochemical reactions can be utilized to identify the enzymatic and metabolic characteristic features of microbes. Microorganisms can be categorically differentiated based on their utilization of specific biochemicals as nutrients during the process of metabolism and/or enzymatic reactions. The specific biochemical, which showed some changes against *S. flexneri* after biofield treatment are shown in Table 3. Biochemicals such as cetrimide (CET), cephalothin (CF8), citrate (CIT), nitrofurantoin (FD64), kanamycin (K4), lysine (LYS) and ornithine (ORN) were changed from negative (-) to positive (+) reaction in Gr. II on day 10 while remain unaltered *i.e.* negative (-) reaction on day 5 in Gr. II and on day 10 in Gr. III with respect to control. Moreover, indole (IND) was changed from positive (+) to negative (-) reaction in Gr. III while remained unchanged *i.e.* positive (+) reaction in Gr. II on both day 5 as well as day 10 with respective to control. Overall, 24.24% (eight out of thirty three) biochemical reactions were altered in tested biochemicals with respect to control after biofield treatment. Revived treated cells of *S. flexneri* (Gr. II) showed 21.21% and lyophilized treated cells (Gr. III) showed 3.03% alteration on day 10 while no alteration was observed on day 5 in Gr. II in term of biochemical reactions as compared to control. About 75.76% (out of thirty three) tested biochemicals, such as acetamide (ACE), adonitol (ADO), arabinose (ARA), arginine (ARG), colistin (CL4), esculin hydrolysis (ESC), glucose (GLU), hydrogen sulfide (H_2S), inositol (INO), malonate (MAL), melibiose (MEL), nitrate (NIT), oxidation-fermentation/glucose (OF/G), ortho-nitrophenyl-β-galactoside (ONPG), oxidase (OXI), penicillin (P4), raffinose (RAF), rhamnose (RHA), sorbitol (SOR), sucrose (SUC), tartrate (TAR), tryptophan deaminase (TDA), tobramycin (TO4), urea (URE) and Voges-Proskauer (VP) did not show any change in all the treated groups after biofield treatment as compared to control (Table 3).

Based on existing literature differentiation of specific *Shigella* serotype on the basis of their sugar fermentation pattern is difficult. The key characteristic feature for *S. flexneri* bacterium is non-lactose fermenting, but it can ferment glucose with production of acid [2].

S. No.	Antimicrobial	Type of Response			
		Gr. I	Gr. II		Gr. III
			Day 5	Day 10	Day 10
1	Amoxicillin/k-clavulanate	S	S	R	S
2	Ampicillin/sulbactam	S	S	R	S
3	Ampicillin	S	S	R	S
4	Aztreonam	S	S	R	S
5	Cefepime	S	S	S	S
6	Cefotaxime	S	S	S	S
7	Ceftazidime	S	S	I	S
8	Ceftriaxone	S	S	S	S
9	Chloramphenicol	S	S	R	S
10	Ciprofloxacin	S	S	S	S
11	Gatifloxacin	S	S	S	S
12	Imipenem	S	S	S	S
13	Levofloxacin	S	S	S	S
14	Meropenem	S	S	S	S
15	Moxifloxacin	S	S	S	S
16	Piperacillin/tazobactam	S	S	S	S
17	Piperacillin	S	S	S	S
18	Tetracycline	S	S	S	S
19	Ticarcillin/k-clavulanate	S	S	S	S
20	Trimethoprim/sulfamethoxazole	S	S	R	S

R: Resistant; I: Intermediate; S: Susceptible; Gr.: Group

Table 1: Antimicrobial susceptibility pattern of *Shigella flexneri*: Effect of biofield treatment.

S. No.	Antimicrobial	Type of Response			
		Gr. I	Gr. II		Gr. III
			Day 5	Day 10	(Day 10)
1	Amikacin	≤16	≤16	≤16	≤16
2	Amoxicillin/k-clavulanate	≤8/4	≤8/4	>16/8	≤8/4
3	Ampicillin/sulbactam	≤8/4	≤8/4	>16/8	≤8/4
4	Ampicillin	≤8	≤8	>16	≤8
5	Aztreonam	≤8	≤8	>16	≤8
6	Cefazolin	≤8	≤8	>16	≤8
7	Cefepime	≤8	≤8	≤8	≤8
8	Cefotaxime	≤8	≤8	≤8	≤8
9	Cefotetan	≤16	≤16	>32	≤16
10	Cefoxitin	≤8	≤8	>16	≤8
11	Ceftazidime	≤8	≤8	16	≤8
12	Ceftriaxone	≤8	≤8	≤8	≤8
13	Cefuroxime	≤4	≤4	>16	≤4
14	Cephalothin	≤8	≤8	>16	≤8
15	Chloramphenicol	≤8	≤8	>16	≤8
16	Ciprofloxacin	≤1	≤1	≤1	≤1
17	ESBL-a Scrn	≤4	≤4	>4	≤4
18	ESBL-b Scrn	≤1	≤1	>1	≤1
19	Gatifloxacin	≤2	≤2	≤2	≤2
20	Gentamicin	≤4	≤4	≤4	≤4
21	Imipenem	≤4	≤4	≤4	≤4
22	Levofloxacin	≤2	≤2	≤2	≤2
23	Meropenem	≤4	≤4	≤4	≤4
24	Moxifloxacin	≤2	≤2	≤2	≤2
25	Nitrofurantoin	≤32	≤32	>64	≤32
26	Norfloxacin	≤4	≤4	≤4	≤4
27	Piperacillin/tazobactam	≤16	≤16	≤16	≤16
28	Piperacillin	≤16	≤16	≤16	≤16
29	Tetracycline	≤4	≤4	≤4	≤4
30	Ticarcillin/k-clavulanate	≤16	≤16	≤16	≤16
31	Tobramycin	≤4	≤4	≤4	≤4
32	Trimethoprim/sulfamethoxazole	≤2/38	≤2/38	>2/38	≤2/38

MIC data are presented in µg/mL; Gr.: Group; ESBL-a, b Scrn: Extended-spectrum β- lactamase screen a and b

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

In this experiment, control sample of *S. flexneri* resulted positive (+) reaction in GLU and negative reaction (-) in case of SUC. The findings were also reported in the literature [29]. These findings could be due to fermentation of GLU and produce acid, which supports the characteristic feature of *S. flexneri*. In the present study, negative reactions (-) of VP and URE utilization tests were observed in control sample of *S. flexneri*. The findings were also reported in the literature [30].

Identification of Organism by Biotype Number

The species (*S. flexneri*) was identified based on variety of conventional biochemical characters and biotyping. Biotype number of particular organism was evaluated after interpreting the results of the biochemical reactions. The biotype number then led to the particular organism identification. Based on the biochemical results, biotype number was changed in revived treated cells (Gr. II) on day 10 (40015042) as compared to untreated strain of *Shigella* species (40010000). In Gr. II due to change in biotype number a new organism *i.e. Edwardsiella tarda* was identified, which could be due to change in enzymatic and/or genetic level after biofield treatment. However, the biotype numbers of *S. flexneri* were not altered in Gr. II (on day 5) and Gr. III (on day 10) as compared to control (Table 4).

S. No.	Code	Biochemical	Type of Response			
			Gr. I	Gr. II		Gr. III
				Day 5	Day 10	Day10
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	-	-	-	-

'-' (Negative); '+' (Positive); Gr.: Group; ONPG: Ortho-nitrophenyl-β-galactoside

Table 3: Effect of biofield treatment on *Shigella flexneri* to the biochemical reaction pattern.

Feature	Gr. I	Gr. II		Gr. III
		Day 5	Day 10	Day 10
Biotype	40010000	40010000	40015042	40000000
Organism Identification	*Shigella* species	*Shigella* species	Edwardsiella tarda	*Shigella* species
Gr.: Group				

Table 4: Effect of biofield treatment on biotype number of *Shigella flexneri*.

Rapid emergence and outbreaks of resistant microorganisms due to widespread selective pressure and efficient dissemination channels are one of the factors that might have contributed to the spread of resistant organisms [31]. Due to microbial resistance to a single or multiple drugs, invention of an effective antimicrobial therapy for the human-wellness is urgently required. However, due to some limitation of science, the progress of new medications are slow and very challenging for scientists. Biofield treatment could be responsible for alteration in microorganism at genetic and/or enzymatic level, which may act on receptor protein. While altering receptor protein, ligand-receptor/protein interactions may alter that could lead to show different phenotypic characteristics [32]. Moreover, the alteration in susceptibility patterns may be due to mutation occurs at genetic level on biofield energy treated *S. flexneri* [33]. Biofield treatment might induce significant changes in revived strain of *S. flexneri* and alter antimicrobials susceptibility pattern, MIC values and biochemical reactions. Based on these results, it is postulated that, biofield treatment could be used to alter the sensitivity pattern of antimicrobials.

Conclusions

Altogether, the biofield treatment has significantly altered the susceptibility pattern (35%) and MIC values (46.88%) of tested antimicrobials against the ATCC strain of *S. flexneri* in revived treated cells (Gr. II) on day 10 as compared to control. It also altered the biochemical reactions pattern (21.21%) in biofield treated strain of *S. flexneri* in Gr. II as compared to control. On the basis of changed biochemical reactions of *S. flexneri* the biotype number was altered in Gr. II with identification of new organism, *Edwardsiella tarda* (40015042) as compared to untreated strain of *Shigella* species (40010000). Mr. Trivedi's biofield treatment could be applied as an alternative therapeutic approach to alter the sensitivity pattern of antimicrobials in near future. These findings suggest that there is a need to carry out extensive susceptibility studies at molecular level.

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