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Effect of Biofield Energy Treatment on *Streptococcus* group B: A Postpartum Pathogen

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Abstract

Streptococcus agalactiae group B (*S. agalactiae* gr. B) is widespread in nature mainly causes bacterial septicemia and neonatal meningitis. The current study was attempted to investigate the effect of biofield treatment on *S. agalactiae* gr. B with respect of antimicrobial sensitivity, biochemical reactions and bio typing. *S. agalactiae* gr. B strain was used in this experiment bearing the American Type Culture Collection (ATCC 12386) number and stored according to the recommended storage protocol. The revived and lyophilized state of ATCC strains of *S. agalactiae* gr. B were selected for the study. Gr. I was considered as control. Both revived (Group; Gr. II) and lyophilized (Gr. III) strains of *S. agalactiae* gr. B were subjected to Mr. Trivedi's biofield treatment. Gr. II was assessed on day 5 and day 10 while Gr. III on day 10 with respect to the control (Gr. I) using MicroScan Walk-Away® system. Although biofield treatment did not show any change with respect to susceptibility pattern. However the minimum inhibitory concentration of *S. agalactiae* gr. B showed significant (70.37%) alteration, out of twenty-seven tested antimicrobials, among which in Gr. II *i.e.* 62.96% on day 5 and 66.67% on day 10 while no alteration was found in lyophilized group (Gr. III) as compared to the control. Moreover, the improvement of MIC value of norfloxacin was observed by two-fold (8 to ≤4 µg/mL) in Gr. II on day 10 after biofield energy treatment as compared to the control. It was observed that overall 48.28% biochemical reactions, out of twenty-nine were altered in Gr. II with respect to the control. Moreover, biotype numbers were changed in Gr. II on day 5 (777777615) and on day 10 (757677405) as compared to the control (237147047). The results suggest that biofield treatment has significant impact on *S. agalactiae* gr. B in revived treated cells (Gr. II) with respect to MIC values, biochemical reactions pattern and biotype number.

Keywords: *Streptococcus agalactiae* group B; Biofield treatment; Minimum inhibitory concentration; Biochemical reaction; Biotype

Abbreviations: CLSI: Clinical and laboratory standards institute; GBS: Group B Streptococci; CAMP: Christie-Atkins-Munch-Petersen; CDC: Centers for Disease Control and Prevention; ACOG: American College of Obstetricians and Gynecologists; AAP: American Academy of Pediatrics; MIC: Minimum inhibitory concentration; ATCC: American Type Culture Collection; PBPC 20: Positive Breakpoint Combo 20

Introduction

Group B Streptococci (GBS), or *Streptococcus agalactiae* group B, are Gram-positive, non-spore forming, non-motile, β-hemolytic and chain-forming cocci bacteria. It is mainly inhabitant in human gut flora and female urogenital tract [1]. Pregnant women [2] and neonates [3] are the main victim host of this organism. It can be transferred to neonates through the birth canal and causes bacterial septicaemia and neonatal meningitis [4]. Most of GBS, produce Christie-Atkins-Munch-Petersen (CAMP) factor or protein B an extracellular cytolytic protein and β-lysin from *Staphyllococcus* species are jointly lysed the erythrocytes [5]. It is estimated that in US over 70,000 cases of GBS diseases were prevented [6]. Several antibiotics such as penicillin, ampicillin, amoxicillin, cephalosporins (all three generations), macrolides, clindamycin and vancomycin (as alternative) have been used to treat against GBS infections. Among above mentioned antibiotics penicillin is the drug of choice next to cefazolin. However, it also have certain limitations such as high minimum inhibitory concentration (MIC), a factor associated with high level of bacteremia and more concentration of microbes in tissue, especially in cerebrospinal fluid [7,8]. Therefore, some alternative treatment strategies are needed to overcome these lacunas against β-hemolytic strain of gr. B Streptococci. Biofield treatment has been known as an alternative approach that may be useful for *S. agalactiae* group B infected patients.

Researchers have shown that short-lived electrical events or action potential exist in the several type of mammalian cells such as neurons, muscles, and endocrine cells [9]. For instance, when the cells present in central nervous system of human body communicate with each another by means of electrical signals that propagate along the nerve impulses. Therefore, it was hypothesized that biofield exists around the human body and evidence was found using electromyography, electrocardiography and electroencephalogram [10]. Thus, the human body emits the electromagnetic waves in the form of bio-photons, which surrounds the body and it is commonly known as biofield. Therefore, the biofield consists of electromagnetic field, being generated by moving electrically charged particles (ions, cell, molecule etc.) inside the human body. Rivera-Ruiz reported that electrocardiography has been extensively used to measure the biofield of human body [11]. 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

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biofield treatment that is also called as Trivedi effect®. Mr. Trivedi's unique biofield treatment has been known to transform the structural, physical, and thermal properties of several metals in material science [12-14], improved the overall productivity of crops [15,16], altered characteristics features of microbes [17-19] and improved growth and anatomical characteristics of various medicinal plants [20,21].

Due to the clinical significance of this organism and literature reports on biofield treatment, the present work was undertaken to evaluate the impact of biofield treatment on *S. agalactiae* group B in relation to antimicrobials susceptibility and bio typing based on various biochemical characters.

Materials and Methods

S. agalactiae group B, American Type Culture Collection (ATCC 12386) 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. All the tested antimicrobials and biochemicals were procured from Sigma-Aldrich, MA, USA. The antimicrobial susceptibility, biochemical reactions and biotype number were estimated with the help of MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA, USA) using Positive Breakpoint Combo 20 (PBPC 20) panel with respect to the control group.

Experimental design

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

Biofield treatment strategy

The Gr. I remained as untreated. The treatment Gr. II and III in sealed pack were handed over to Mr. Trivedi for biofield energy 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. agalactiae* gr. B was carried out with the help of automated instrument, MicroScan Walk-Away® system using PBPC 20 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. agalactiae* gr. B 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 and MIC were determined by observing the lowest antimicrobial concentration showing growth inhibition [22].

Biochemical reaction studies

Biochemical reactions of *S. agalactiae* gr. B were determined using MicroScan Walk-Away®, system with PBPC 20 panel [22]. Biochemicals used in the study were arabinose, arginine, bacillosamine, bile esculin, β-lactamases, crystal violet, hemolysin, indoxyl phosphatase, inulin, acidification lactose, mannitol, mannose, micrococcus screen, sodium chloride, nitrate, novobiocin, optochin, *p*-nitro phenyl β-D-glucuronide, *p*-nitro phenyl β-D-galactopyranoside, phosphatase, pyruvate, pyrolidonyl arylamidase, raffinose, rambose, sorbitol, thymidine free growth, acidification trehalose, urea, and Voges-Proskauer.

Identification of organism by biotype number

The biotype number of *S. agalactiae* gr. B was determined on MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data [22].

Results and Discussion

Antimicrobial susceptibility test

The outcomes of MIC values of tested antimicrobials against *S. agalactiae* gr. B after biofield treatment are summarized in Table 1. The data were analyzed using automated system and compared with respect to the control. In this experiment, twelve antibiotics were used to investigate the susceptibility pattern on GBS *viz.* ampicillin, cefepime, cefotaxime, ceftriaxone, chloramphenicol, clindamycin, levofloxacin, linezolid, ofloxacin, penicillin, tetracycline, and vancomycin. Out of these, five antibiotics showed susceptible in the control sample. It has been evidenced from literatures that penicillin and its derivatives are the choice of drugs against gr. B streptococcal infection [8,9]. However, biofield treatment did not show any alteration with respect to susceptibility pattern in all the treated groups after biofield treatment (data not shown). Besides sensitivity assay, the MIC values of tested antimicrobials were significantly (70.37%) altered out of twenty seven as compared to the control. The MIC value of norfloxacin was reduced by two-fold to ≤ 4 µg/mL after biofield treatment in Gr. II on day 10 as compared to the control (8 µg/mL). The MIC values of cefazolin, cephalothin, and chloramphenicol were changed from ≤ 8 to >16 µg/mL in Gr. II on day 5 and 10 as compared to the control. The MIC values of rifampin and synercid were changed from ≤ 1 to >2 µg/mL in Gr. II on day 5 and 10 as compared to the control. Alteration of MIC values of cefotaxime and ceftriaxone were changed from ≤8 to 32 µg/mL in Gr. II on day 5 and 10 as compared to the control. Moreover, MIC values of amoxicillin/k-clavulanate and trimethoprim/sulfamethoxazole were slightly changed from ≤4/2 to >4/2 µg/mL (on day 5 and 10) and ≤2/38 to >2/38 µg/mL (on day 5) respectively in Gr. II after biofield treatment as compared to the control. Antimicrobial linezolid showed an alteration of MIC value in Gr. II on day 5 (>4 µg/mL) and on day 10 (4 µg/mL) as compared to the control. Besides this, alteration of MIC values were observed in case of penicillin (≤ 0.03 to >8 µg/mL), vancomycin (≤ 0.2 to >16 µg/mL), nitrofurantoin (≤ 32 to >64 µg/mL), clindamycin (≤ 0.5 to >2 µg/mL), ampicillin (≤ 0.25 to >8 µg/mL) and ampicillin/sulbactam (≤ 8/4 to >16/8 µg/mL) in Gr. II on day 5 and 10 as compared to the control. The MIC value of tetracycline was changed from ≤ 4 to 8 µg/mL in Gr. II on day 10 and MIC value of oxacillin was changed from ≤ 0.25 to >2 µg/mL (on day 5) and 2 µg/mL on day 10 in Gr. II as compared to control. Antimicrobials did not show any change in MIC value in Gr. III as compared to the control after biofield treatment. Seventeen out of twenty seven (62.96%) antimicrobials showed alteration of MIC value in Gr. II on day 5 and 66.67% (eighteen

out of twenty seven) on day 10 as compared to the control after biofield treatment. Eight, out of twenty seven tested antimicrobials (29.63%) *viz.* cefepime, ciprofloxacin, gatifloxacin, imipenem, levofloxacin, moxifloxacin, ofloxacin and piperacillin/tazobactam did not show any alteration of MIC values in all the treated cells of GBS as compared to the control (Table 1).

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 or enzymatic reactions. The specific biochemical showed some changes against *S. agalactiae* gr. B after biofield treatment that are shown in Table 2. Biochemicals such as bile esculin (BE), crystal violet (CV), mannitol (MAN), sodium chloride (NaCl), nitrate (NIT), novobiocin (NOV), pyrolidonyl arylamidase (PYR) and urea (URE) were changed from negative (-) to positive (+) reactions in revived treated group (Gr. II) on day 5 and 10, but remained same *i.e.* negative (-) reaction in lyophilized treated cells (Gr. III) with respective to control. Arginine (ARG) and glycosidase (PGR) were converted from positive (+) to negative (-) reactions in Gr. II on day 10, while remained unchanged *i.e.* positive (+) on day 5 in Gr. II and in Gr. III as compared to the control in biofield treated *S. agalactiae* gr. B. Similarly, pyruvate (PRV), and rambose (RBS) were converted from positive (+) to negative (-) reactions on both days in Gr. II, while did not show any change in Gr. III as compared with the control. Moreover, biochemicals such as raffinose (RAF) and sorbitol (SOR) showed an alteration of biochemical reaction *i.e.* negative (-) to positive (+) in Gr. II on day 5 while remained negative in Gr. II on day 10 and in Gr. III as compared to the control.

The one of the key characteristic feature for GBS is complete lysis of red blood cells and metabolization of sugars by fermentation process. Hence, in control sample the positive reaction of hemolysin (HEM) indicated β-hemolysis of erythrocytes and positive reaction of acidifying lactose (LAC) indicated the production of lactic acid as bi-product. The data was supported with literature [23]. Similarly, based on existing literature pyrolidonyl arylamidase (PYR) has negative reaction in group B streptococcal species while positive reaction in group A streptococcal. Control data of PYR was well supported with literature data [24]. However, after biofield treatment the negative reaction was altered in Gr. II, assessed on day 5 as well as day 10 due to change of enzymatic reaction.

Overall, 48.28% biochemical reactions were altered in tested twenty-nine biochemicals with respect to the control after biofield treatment. In both time points of Gr. II (day 5 and 10) 41.38% (twelve out of twenty-nine) biochemical reactions were altered as compared to the control. About 51.72% out of twenty-nine biochemicals, such as arabinose (ARA), bacillosamine (BAC), β-lactamase (BL), hemolysin (HEM), indoxyl phosphatase (IDX), inulin (INU), acidification lactose (LAC), mannose (MNS), micrococcus screen (MS), optochin (OPT), glycosidase (PGT), phosphatase (PHO), thymidine free growth (TFG), acidification trehalose (TRE), and Voges-Proskauer (VP) did not

S. No.	Antimicrobial	Type of Response			
		Gr. I	Gr. II		Gr. III
			Day 5	Day 10	
1.	Amoxicillin/k-clavulanate	≤4/2	>4/2	>4/2	≤4/2
2.	Ampicillin/sulbactam	≤8/4	>16/8	>16/8	≤8/4
3.	Ampicillin	≤0.25	>8	>8	≤0.25
4.	Cefazolin	≤8	>16	>16	≤8
5.	Cefepime	≤8	≤8	≤8	≤8
6.	Cefotaxime	≤8	32	32	≤8
7.	Ceftriaxone	≤8	32	32	≤8
8.	Cephalothin	≤8	>16	>16	≤8
9.	Chloramphenicol	≤8	>16	>16	≤8
10.	Ciprofloxacin	≤1	≤1	≤1	≤1
11.	Clindamycin	≤0.5	>2	>2	≤0.5
12.	Gatifloxacin	≤2	≤2	≤2	≤2
13.	Imipenem	≤4	≤4	≤4	≤4
14.	Levofloxacin	≤2	≤2	≤2	≤2
15.	Linezolid	≤2	>4	4	≤2
16.	Moxifloxacin	≤2	≤2	≤2	≤2
17.	Nitrofurantoin	≤32	>64	>64	≤32
18.	Norfloxacin	8	8	≤4	8
19.	Ofloxacin	≤2	≤2	≤2	≤2
20.	Oxacillin	≤0.25	>2	2	≤0.25
21.	Penicillin	≤0.03	>8	>8	≤0.03
22.	Piperacillin/tazobactam	≤4	≤4	≤4	≤4
23.	Rifampin	≤1	>2	>2	≤1
24.	Synercid	≤1	>2	>2	≤1
25.	Tetracycline	≤4	≤4	8	≤4
26.	Trimethoprim/sulfamethoxazole	≤2/38	>2/38	≤2/38	≤2/38
27.	Vancomycin	≤2	>16	>16	≤2

MIC values are presented in µg/mL; Gr.: Group

Table 1: Effect of biofield treatment on *Streptococcus agalactiae* group B to minimum inhibitory concentration (MIC) of tested antimicrobials.

S. No.	Code	Biochemical	Gr. I	Type of Response		
				Gr. II		Gr. III
				Day 5	Day 10	
1.	ARA	Arabinose	-	-	-	-
2.	ARG	Arginine	+	+	-	+
3.	BAC	Bacillosamine	+	+	+	+
4.	BE	Bile esculin	-	+	+	-
5.	BL	Beta lactamases	NR	NR	NR	NR
6.	CV	Crystal violet	-	+	+	-
7.	HEM	Hemolysin	+	+	+	+
8.	IDX	Indoxyl phosphatase	+	+	+	+
9.	INU	Inulin	-	-	-	-
10.	LAC	Acidification lactose	+	+	+	+
11.	MAN	Mannitol	-	+	+	-
12.	MNS	Mannose	+	+	+	+
13.	MS	Micrococcus screen	+	+	+	+
14.	NaCl	Sodium chloride	-	+	+	-
15.	NIT	Nitrate	-	+	+	-
16.	NOV	Novobiocin	-	+	+	-
17.	OPT	Optochin	+	+	+	+
18.	PGR	Glycosidase*	+	+	-	+
19.	PGT	Glycosidases#	+	+	+	+
20.	PHO	Phosphatase	+	+	+	+
21.	PRV	Pyruvate	+	-	-	+
22.	PYR	Pyrolidonyl arylamidase	-	+	+	-
23.	RAF	Raffinose	-	+	-	-
24.	RBS	Rambose	+	-	-	+
25.	SOR	Sorbitol	-	+	-	-
26.	TFG	Thymidine free growth	+	+	+	+
27.	TRE	Acidification trehalose	+	+	+	+
28.	URE	Urea	-	+	+	-
29.	VP	Voges-Proskauer	+	+	+	+

'-' (negative); '+' (positive); Gr.: Group; NR: Not reported; *PGR: p-nitro phenyl β-D- glucuronide; #PGT: p-nitro phenyl β-D-galactopyranoside.

Table 2: Effect of biofield treatment on *Streptococcus agalactiae* group B to the biochemical reaction pattern.

show any change in all the treated groups after biofield treatment as compared to the control.

Identification of organism by biotype number

The species (*S. agalactiae* gr. B) 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. In this experiment, biotyping was performed using automated systems. Results showed changes of biotype numbers in the biofield treated Gr. II (on day 5 and 10) and Gr. III (on day 10) without alteration of organism. Based on the biochemical data, biotype number was changed in treated Gr. II on day 5 (777777615, *S. agalactiae* group B) and on day 10 (757677405, *S. agalactiae* group B) with respect to the control (237147047) *i.e. S. agalactiae* group B (Table 3). Biotyping, covers the overall cellular pattern of enzymatic activities of any organism. GBS can be characterized based on analysis of biochemical properties with CAMP, Na-hippurate positive and esculin negative as key characters [25]. In this experiment, bile esculin also showed negative (-) reaction in control sample, that directly correlated with literature. This negative reaction of esculin was altered after biofield treatment in revived treated cells of GBS. It was indicated that biofield treatment has the ability to alter the biochemical pattern which may be due to change the enzymatic reaction. So, it is assumed that these changes of biotype number without alteration in organism may be due to change of metabolic and/or enzymatic reactions of GBS.

Biofield treatment may responsible for alteration in microorganism at genetic and/or enzymatic level, which probably act on receptor protein. While altering receptor protein, ligand-receptor/protein interactions may alter that could lead to different phenotypic characteristics [26]. Biofield treatment might induce a significant changes in revived strain of GBS and altered the MIC values, biochemical reactions, and ultimately change the biotype number of microorganism.

Conclusion

Altogether, the biofield treatment has significantly altered 70.37%, (out of twenty-seven) the MIC values of tested antimicrobials against the strain of *S. agalactiae* gr. B. Norfloxacin was improved the MIC value by two-fold (8 to ≤4 μg/mL) in Gr. II on day 10 after biofield energy treatment as compared to the control. Additionally, it also significantly (48.28%) altered the biochemical reactions pattern of biofield energy treated strain of *S. agalactiae* gr. B. On the basis of utilization of group B streptococcal specific biochemicals, change in metabolic reactions led to variation of biotype number in all the treated groups without change of organism after biofield treatment with respect to the control. Based on above findings, it is assumed that Mr. Trivedi's biofield treatment is

Feature	Gr. I	Gr. II		Gr. III
		Day 5	Day 10	Day 10
Biotype	237147047	777777615	757677405	237147047
Organism Identification	*Streptococcus agalactiae* group B	*Streptococcus agalactiae* group B (Very rare biotype)	*Streptococcus agalactiae* group B (Very rare biotype)	*Streptococcus agalactiae* group B

Gr.: Group

Table 3: Effect of biofield treatment on biotype number of Streptococcus agalactiae group B.

an alternative approach to alter the antibiogram profile of *S. agalactiae* gr. B.

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