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Antibiogram, Biochemical Reactions and Biotyping of Biofield Treated *Providencia rettgeri*

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Abstract: *Providencia rettgeri* (*P. rettgeri*) is the key organism for gastrointestinal tract infections due to its high virulence properties. The current study was designed to investigate the effect of Mr. Trivedi's biofield energy treatment on *P. rettgeri* in lyophilized as well as revived state for antimicrobial susceptibility pattern, biochemical characteristics, and biotype number. The lyophilized strain of *P. rettgeri* (ATCC 9250) was divided into two parts, Group (Gr.) I: control and Gr. II: treatment. After biofield treatment, Gr. II was further subdivided into two parts, Gr. IIA and Gr. IIB. Gr. IIA was analyzed on day 10, while Gr. IIB was stored and analyzed on day 162 after revival (Study I). The revived sample of Gr. IIB was retreated on day 162 (Study II), and divided into three separate tubes. Tube 1 was analyzed on day 5, likewise, tube 2 and 3 were analyzed on day 10 and 15, respectively after their sub-culturing. All the experimental parameters were studied using automated MicroScan Walk-Away® system. The antimicrobial susceptibility and minimum inhibitory concentration were significantly improved by 71.43%, out of twenty-eight and 56.25%, out of thirty-two, respectively in the treated cells of *P. rettgeri* as compared to the control. The biochemical reactions also showed the significant (60.61%) alteration in the treated sample with respect to control. The biotype numbers were substantially changed in all the treated groups as compared to the control. Moreover, the organism was changed as *Proteus mirabilis* in all the treated groups except in Gr. IIA, as compared to the control. These results suggested that biofield treatment has a significant impact on *P. rettgeri* in lyophilized as well as revived state.

Keywords: *Providencia rettgeri,* Antimicrobial Sensitivity, Minimum Inhibitory Concentration, Biofield Treatment, Biochemical Reaction, Biotype

1. Introduction

Providencia rettgeri (*P. rettgeri*) is the key organism for gastrointestinal tract infections due to its high virulence properties. The genus *Providencia* is facultatively anaerobic, chemoorganotrophic, and urease-producing Gram-negative, rod-shaped bacterium that are responsible for a wide spectrum of human infections [1, 2]. *Providencia rettgeri* (*P. rettgeri*) is motile by peritrichous flagella, belonging to the family of *Enterobacteriaceae*. The most remarkable biochemical features to characterize its biochemical abilities are positive reactions of urea and catalase, negative reactions of oxidase, hydrogen sulfide and β-galactosidase [3]. The high abundance of *P. rettgeri* is mainly in the urinary tract of the compromised or catheterized patient that causes gastrointestinal tract infections or traveler's diarrhea [4, 5]. The virulence factors of *P. rettgeri* are lipopolysaccharide (LPS) and production of sidrophores, β-lactamase, and urease [6]. Ciprofloxacin, levofloxacin, piperacillin-tazobactam, meropenem, and amikacin are the choice of drugs in *P. rettgeri* infection. Based on literature, it was reported that the β-lactamase producing *P. rettgeri* had marked resistance to multiple drugs [7]. Therefore, due to the clinical significance of *P. rettgeri*, an effective antimicrobial therapy is very needful for human health. An alternative *i.e.* biofield energy based healing therapy is recently reported to alter the antimicrobial sensitivity pattern in a different microorganism. Biofield (putative energy fields) or electromagnetic based energy therapies used to promote health and healing had exclusively reported by National Institute of Health/National Center for Complementary and

Alternative Medicine (NIH/NCCAM) [8]. The human body naturally emits the waves in the form of bio-photons, which surrounds the body and it is commonly known as biofield. Therefore, the biofield consists of an electromagnetic field, being generated by moving electrically charged particles such as ions, molecule, etc. inside the human body. In the recent year, 2015 Prakash *et al.* reported that the various scientific instruments such as Kirlian photography, polycontrast interference photography (PIP) and resonance field imaging (RFI) can be extensively used to measure the biofield of human body [9]. Although, a human has the capability to harness the energy from environment or universe and can transmit it into any object(s) around the Globe. The objects always receive the energy and respond it into a useful way that is called biofield energy and the process is known as biofield treatment. Mr. Trivedi's unique biofield energy treatment (The Trivedi Effect®) has been known to alter the characteristics features of pathogenic microbes [10, 11], an improved growth and productivity of plants [12, 13] and also able to alter the thermophysical properties of metal and ceramic in materials science [14, 15].

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 modality on *P. rettgeri* in relation to the antimicrobials susceptibility, biochemical reactions, and biotyping.

2. Materials and Methods

The strain *P. rettgeri*, bearing the American Type Culture Collection (ATCC 9250) strain was procured from MicroBioLogics, Inc., USA. All the antimicrobial agents and biochemicals used in this experiment were procured from Sigma-Aldrich, MA, USA. The antimicrobial susceptibility, biochemical reactions and biotype number were estimated using MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA, USA) with Negative Breakpoint Combo 30 (PBPC 30) panel.

2.1. Experimental Design

The impact of biofield treatment on tested bacterium *P. rettgeri* was evaluated in two groups-

Group I: ATCC strain in the lyophilized state was considered as control. No treatment was given and the group was analyzed for antimicrobial sensitivity, biochemical reactions and biotype number as per the standard protocol.

Group II: The lyophilized state of ATCC strain was divided into two parts named as Gr. IIA and Gr. IIB. Both the groups of ATCC strain of *P. rettgeri* in the lyophilized state were subjected to the Mr. Trivedi's unique biofield treatment (first treatment). Gr. IIA was analyzed on day 10 for antimicrobial sensitivity, MIC, biochemical reactions and biotyping were performed as per the standard protocol, while Gr. IIB sample was stored in the lyophilized state for 162 days at -70ºC. Gr. IIB was further sub-divided in two separate parts named as Gr. IIB - Study I and Gr. IIB - Study II.

Group IIB - Study I

After 162 days, antimicrobial sensitivity, MIC, biochemical reactions and biotyping were performed as per the standard protocol.

Group IIB - Study II

The stored strain was revived from -70ºC and the revived culture was again subjected to Mr. Trivedi's biofield treatment (re-treatment) on day 162. After biofield retreatment, the sample was sub-cultured into three separate tubes on three different days (Day 0, Day 5 and Day 10) and were analysed keeping the main treated tube aside. Each sample was analyzed after five days of its sub-culturing.

2.2. Biofield Treatment Strategy

The lyophilized sample of *P. rettgeri* was subjected to Mr. Trivedi's biofield treatment (first treatment) and then stored, analyzed on day 10 (Gr. IIA) followed by retreatment on 162 days in revived state (Gr. IIB, Study II) for antimicrobial sensitivity along with minimum inhibitory concentration (MIC), biochemical reactions and biotype number as per the standard protocol. In details, the treatment groups were received to Mr. Trivedi's biofield treatment in sealed pack under laboratory condition. Mr. Trivedi provided the treatment through his energy transmission process to the treated groups without touching the samples. The optimum precautions were taken while handing over these cultures to Mr. Trivedi for retreatment purposes, to avoid contamination.

2.3. Antimicrobial Susceptibility Test

The assessment of antimicrobial sensitivity of *P. rettgeri* was carried out using automated instrument, MicroScan Walk-Away® with NBPC 30 panel. The panel was stored at 2 to 25ºC for analysis. The panel was allowed to equilibrate to room temperature before rehydration. All opened panels were used on the same day. The tests carried out on MicroScan were miniaturized of the broth dilution susceptibility test that has been dehydrated. Briefly, 0.1 mL of the standardized suspension of *P. rettgeri* cultured cells were pipetted into 25 mL of inoculum water using pluronic and inverted 8 to 10 times and inoculated, rehydrated, and then subjected to incubation for 16 hours at 35°C. Rehydration and inoculation were performed using the RENOK® system with inoculators-D (B1013-4). Approximately 25 mL of standardized inoculum suspension was poured into the inoculum tray. The detailed experimental procedure and conditions were maintained as per the manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate; R: Resistant; and IB: Inducible β-lactamase positive) and MIC were determined by observing the lowest antimicrobial concentration that inhibits the growth of microbes [16].

2.4. Biochemical Reaction Studies

The biochemical reactions of *P. rettgeri* were determined using MicroScan Walk-Away® system with NBPC 30 panel. Preparation of NBPC 30 panel, inoculum followed by dehydration and rehydration were performed in a similar way

as mentioned in antimicrobial susceptibility assay for analysis of biochemical reactions followed by biotype number. It interprets the microbe's biochemical results with the use of a photometric or fluorogenic reader. On the basis of nature of bacilli (Gram-negative or Gram-positive), it generates computerized reports using conventional panels, which utilizes the photometric reader and provide identification results. Before commencing the experiment, the NBPC 30 panel was first incubated and read on the MicroScan Walkaway system. Then the panel was removed from the system and read on the Biomic system within 1 hour. MicroScan Walk-Away instrument consist of a database associated with collective information, which is essential to identify the group, genera, or species of the family. The detailed experimental procedures and conditions were followed as per the manufacturer's instructions [16].

2.5. Identification of Organism by Biotype Number

The biotype number of *P. rettgeri* was determined on MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data. Similar experimental procedure was followed for identification of biotype number as described in biochemical reaction study, and as per manufacturer-recommended instructions [16].

3. Results and Discussion

3.1. Antimicrobial Susceptibility Test

The results of *P. rettgeri* susceptibility pattern and MIC values of tested antimicrobials after biofield treatment are shown in Table 1 and 2, respectively. The data was assessed and compared with respect to the control. Antimicrobial sensitivity assay and MIC were performed in twenty-eight and thirty-two antimicrobials, respectively. Overall, the treated cells of *P. rettgeri* showed a significant (78.57%) alteration (twenty-two out of twenty-eight) in antimicrobial susceptibility pattern as compared with the control. The sensitivity pattern of antibiotics such as amikacin and cefepime were converted from resistance (R) to susceptible (S) in all the treated groups with respect to the untreated control group. Several antibiotics *viz.* aztreonam, cefotaxime, cefotetan, cefoxitin, ceftazidime, cefuroxime, and cephalothin were changed from R to inducible β-lactamase (IB) in lyophilized treated groups (Gr. IIB; on day 10 and Gr. IIB; Study I - on day 162), while R to S in revived treated group (Gr. IIB; Study II) in all three days with respect to control. Amoxicillin/k-clavulanate ceftriaxone and piperacillin/tazobactam were converted from IB to S in Gr. IIB; Study II in all three assessment days, while remained unchanged *i.e.* IB in lyophilized treated groups (Gr. IIA and Gr. IIB; Study I) as compared to the control. Tobramycin was reported with improved antimicrobial sensitivity pattern from R (control) to S in all the treated groups except intermediate type response in Gr. IIB; Study II on day 15. The antimicrobial susceptibility of ticarcillin/k-clavulanate showed an improve response from I (control) to IB in lyophilized treated groups (Gr. IIA and Gr. IIB; Study I) and completely susceptible in revived treated group (Gr. IIB; Study II) in all the assessment days. Further, the antimicrobial sensitivity of gentamicin was changed from R to S in Gr. IIA on day 10, while R to I in Gr. IIB; Study I and in revived treated Gr. IIB; Study II on day 162 except R on day 15 as compared to the control (Gr. I). Chloramphenicol showed R to S in Gr. IIA on day 10 while became R in rest of the treated groups as compared to the control. Cefazolin and ampicillin/sulbactam were converted from I to IB in lyophilized treated groups (Gr. IIA and Gr. IIB; Study I) and absolutely S in revived treated Gr. IIB; Study II in all the assessment days with respect to the control. The antimicrobial sensitivity of ampicillin showed R to IB response in Gr. IIA on day 10 while became R in rest of the treated groups as compared to the control. The antimicrobial sensitivity pattern of piperacillin was changed from IB to S in Gr. IIB; Study II on day 5, while IB to R in rest of the treated groups as compared to the control. Besides, moxifloxacin was converted from S to R in Gr. IIB; Study I and II except it showed intermediate (I) response on day 10, while remained same *i.e.* S in Gr. IIA as compared to the control. The antimicrobial susceptibility pattern of sulphamethoxazole/trimethoprim was altered from S to R all the treated groups except in Gr. IIA, on day 10 with respect to untreated cells of *P. rettgeri*. Six out of twenty eight (21.43%) antimicrobials such as ciprofloxacin, gatifloxacin, imipenem, levofloxacin, meropenem, and tetracycline did not show any alteration of sensitivity pattern after biofield treatment in all the treated groups as compared to the control (Table 1).

Besides antimicrobials susceptibility, the MIC value was also reduced in several antimicrobials after biofield energy treatment on *P. rettgeri*.

Certain antimicrobials such as amikacin, ampicillin/sulbactam, aztreonam, cefazolin, cefepime, cefotetan, cefoxitin, ceftazidime, and cephalothin showed two-fold reduction in MIC values in all the treated groups as compared to the control. The control MIC values of cefoxitin (>16 µg/mL) and cephalothin (>16 µg/mL) were well matched with literature reported data [17]. In this experiment, the MIC values of cefoxitin and cephalothin were reduced by two-fold after biofield energy treatment in both lyophilized as well as revived treated groups as compared to the control. The MIC values of antibiotics such as cefotaxime (>32 to ≤8 µg/mL), cefuroxime (>16 to ≤4 µg/mL) and ticarcillin/k-clavulanate (64 to ≤16 µg/mL) were reduced by four-fold in all the treated groups as compared to the control. Moreover, the MIC values of ampicillin and chloramphenicol were decreased by two-fold (>16 to ≤8 µg/mL) in revived treated Gr. IIA on day 10 while remained unaltered in rest of the treated groups as compared to the control. The MIC value of extended spectrum β-lactamase-a Scrn (ESBL-a Scrn) was slightly reduced (>4 to ≤4 µg/mL) in all the treated groups as compared to the control. However, the MIC value of ESBL-b Scrn was also slightly reduced (>1 to ≤1 µg/mL) in all the treated groups while remained same in Gr. IIB; Study 1 on day 162 as compared to the control. Gentamicin showed reduction in MIC value by two-fold (>8 to ≤4 µg/mL) in Gr. IIA on day 10, while slight reduction in MIC value (>8 to

8 µg/mL) in Gr. IIB; Study I and II (on day 5 and 10) while gave similar result in Gr. IIB; Study II on day 15 as compared to the control. Besides, the MIC value of moxifloxacin was altered by two-fold in all the treated groups as compared to the control except in Gr. IIA. Moreover, the MIC value of piperacillin was also altered by four-fold in Gr. IIA, Gr. IIB (Study I) and Gr. IIB; Study II on all three days of analysis except on day 5 as compared to the control (Table 2).

Table 1. *Antibiogram of Providencia rettgeri: Effect of biofield treatment on antimicrobial susceptibility.*

S. No.	Antimicrobial	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 162)	Gr. IIB (Study II; Day 162)		
					Day 5	Day 10	Day 15
1.	Amikacin	R	S	S	S	S	S
2.	Amoxicillin/k-clavulanate	IB	IB	IB	S	S	S
3.	Ampicillin/sulbactam	I	IB	IB	S	S	S
4.	Ampicillin	R	IB	R	R	R	R
5.	Aztreonam	R	IB	IB	S	S	S
6.	Cefazolin	I	IB	IB	S	S	S
7.	Cefepime	R	S	S	S	S	S
8.	Cefotaxime	R	IB	IB	S	S	S
9.	Cefotetan	R	IB	IB	S	S	S
10.	Cefoxitin	R	IB	IB	S	S	S
11.	Ceftazidime	R	IB	IB	S	S	S
12.	Ceftriaxone	IB	IB	IB	S	S	S
13.	Cefuroxime	R	IB	IB	S	S	S
14.	Cephalothin	R	IB	IB	S	S	S
15.	Chloramphenicol	R	S	R	R	R	R
16.	Ciprofloxacin	S	S	S	S	S	S
17.	Gatifloxacin	S	S	S	S	S	S
18.	Gentamicin	R	S	I	I	I	R
19.	Imipenem	S	S	S	S	S	S
20.	Levofloxacin	S	S	S	S	S	S
21.	Meropenem	S	S	S	S	S	S
22.	Moxifloxacin	S	S	R	R	I	R
23.	Piperacillin/tazobactam	IB	IB	IB	S	S	S
24.	Piperacillin	IB	IB	R	S	R	R
25.	Tetracycline	R	R	R	R	R	R
26.	Ticarcillin/k-clavulanate	I	IB	IB	S	S	S
27.	Tobramycin	R	S	S	S	S	I
28.	Sulfamethoxazole/trimethoprim	S	S	R	R	R	R

R: Resistant; S: Susceptible; I: Intermediate; IB: Inducible β-lactamase positive; Gr.: Group

Table 2. *Effect of biofield treatment on Providencia rettgeri to minimum inhibitory concentration (MIC) of tested antimicrobials.*

S. No.	Antimicrobial	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 162)	Gr. IIB (Study II; Day 162)		
					Day 5	Day 10	Day 15
1.	Amikacin	>32	≤16	≤16	≤16	≤16	≤16
2.	Amoxicillin/k-clavulanate	≤8/4	≤8/4	≤8/4	≤8/4	≤8/4	≤8/4
3.	Ampicillin/sulbactam	16/8	≤8/4	≤8/4	≤8/4	≤8/4	≤8/4
4.	Ampicillin	>16	≤8	>16	>16	>16	>16
5.	Aztreonam	>16	≤8	≤8	≤8	≤8	≤8
6.	Cefazolin	16	≤8	≤8	≤8	≤8	≤8
7.	Cefepime	>16	≤8	≤8	≤8	≤8	≤8
8.	Cefotaxime	>32	≤8	≤8	≤8	≤8	≤8
9.	Cefotetan	>32	≤16	≤16	≤16	≤16	≤16
10.	Cefoxitin	>16	≤8	≤8	≤8	≤8	≤8
11.	Ceftazidime	>16	≤8	≤8	≤8	≤8	≤8
12.	Ceftriaxone	≤8	≤8	≤8	≤8	≤8	≤8
13.	Cefuroxime	>16	≤4	≤4	≤4	≤4	≤4
14.	Cephalothin	>16	≤8	≤8	≤8	≤8	≤8
15.	Chloramphenicol	>16	≤8	>16	>16	>16	>16
16.	Ciprofloxacin	≤1	≤1	≤1	≤1	≤1	≤1
17.	ESBL-a Scrn	>4	≤4	≤4	≤4	≤4	≤4
18.	ESBL-b Scrn	>1	≤1	>1	≤1	≤1	≤1
19.	Gatifloxacin	≤2	≤2	≤2	≤2	≤2	≤2
20.	Gentamicin	>8	≤4	8	8	8	>8
21.	Imipenem	≤4	≤4	≤4	≤4	≤4	≤4
22.	Levofloxacin	≤2	≤2	≤2	≤2	≤2	≤2
23.	Meropenem	≤4	≤4	≤4	≤4	≤4	≤4
24.	Moxifloxacin	≤2	≤2	>4	>4	4	>4
25.	Nitrofurantoin	>64	>64	>64	>64	>64	>64

S. No.	Antimicrobial	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 162)	Gr. IIB (Study II; Day 162)		
					Day 5	Day 10	Day 15
26.	Norfloxacin	≤4	≤4	≤4	≤4	≤4	≤4
27.	Piperacillin/tazobactam	≤16	≤16	≤16	≤16	≤16	≤16
28.	Piperacillin	≤16	≤16	>64	≤16	>64	>64
29.	Tetracycline	>8	>8	>8	>8	>8	>8
30.	Ticarcillin/k-clavulanate	64	≤16	≤16	≤16	≤16	≤16
31.	Tobramycin	>8	≤4	≤4	≤4	≤4	8
32.	Trimethoprim/sulfamethoxazole	≤2/38	≤2/38	>2/38	>2/38	>2/38	>2/38

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

Tobramycin showed two-fold reduction in MIC value (>8 to ≤4 µg/mL) in all the treated groups except slight reduction in MIC value (>8 to 8 µg/mL) in Gr. IIB; Study II on day 15 as compared with the control. The MIC value of trimethoprim/sulfamethoxazole was slightly altered in all the treated groups as compared to the control except in Gr. IIA, where it was remained unchanged. Overall, the treated cells of *P. rettgeri* showed a significant (65.63%) alteration (twenty-one out of thirty-two) of MIC values of tested antimicrobials as compared with the control. Eleven out of thirty-two (34.38%) antimicrobials such as amoxicillin/k-clavulanate, ceftriaxone, ciprofloxacin, gatifloxacin, imipenem, levofloxacin, meropenem, nitrofurantoin, norfloxacin, piperacillin/tazobactam, and tetracycline did not show any alteration in MIC in all the treated groups as compared to the control (Table 2). The organism *P. rettgeri* has been identified as virulent human uropathogens causes bacteremia during prolonged urinary catheterization. Based on literature, the organism has resistance to many common antibiotics such as penicillins, tetracyclines, older cephalosporins, and sulfamethoxazole [18]. In this experiment, the resistant pattern of all the tested penicillins and cephalosporins were improved to some extent and simultaneously reduced the MIC by upto four-fold after treatment with bio-energy on *P. rettgeri*. In recent years *P. rettgeri* has considered as a nosocomial pathogen in immunocompromised patients [19].

3.2. Biochemical Reactions Studies

Table 3. *Effect of biofield treatment on Providencia rettgeri to the biochemical reaction pattern.*

S. No.	Code	Biochemical	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 162)	Gr. IIB (Study II; Day 162)		
						Day 5	Day 10	Day 15
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

The data obtained from biochemical reactions studies for the distinction of *P. rettgeri* are illustrated in Table 3. The study of biochemical reactions can be utilized to identify the enzymatic and metabolic characteristic feature of microbes. The microorganism can be categorically differentiated based on their utilization of specific biochemicals as nutrients during the process of enzymatic reactions or metabolism. Based on results from biochemical reaction tryptophan deaminase (TDA) was changed from negative (-) to positive (+) reaction in all the treated groups with respect to the control. Biochemicals such as arabinose (ARA), cephalothin (CF8), lysine (LYS), malonate (MAL), melibiose (MEL), galactosidase (ONPG), raffinose (RAF), rhamnose (RHA), sorbitol (SOR), and sucrose (SUC) were changed from positive (+) to negative (-) reactions in all the treated groups with respect to the control. Moreover, biochemical reactions of adonitol (ADO), esculin hydrolysis (ESC), and inositol (INO) were changed from positive (+) to negative (-) reactions in all the treated groups while remained unchanged *i.e.* positive (+) in Gr. IIA as compared to the control. Biochemicals such as kanamycin (K4), ornithine (ORN), and Voges-Proskauer (VP) were converted from positive (+) to negative (-) reactions in Gr. IIA on day 10 while remained positive (+) reaction in rest of the treated groups as compared to the control. Hydrogen sulfide (H_2S) and tobramycin (TO4) showed negative (-) reactions in all the treated groups, while H_2S and TO4 gave positive (+) reactions in Gr. IIB; Study II on day 10 and 15, respectively as compared with the control. The positive (+) reaction of H_2S in control sample is the key characteristic feature of *P. rettgeri* which was altered after biofield treatment. Moreover, cetrimide (CET) showed the positive (+) reaction in Gr. IIB; Study II on day 5 and 15, while remained same as a negative (-) reaction in rest of the treated groups as compared to the control. Based on this data, it is assumed that Mr. Trivedi's biofield treatment has an impact on *P. rettgeri* in terms of metabolic reaction. Overall, 60.61% (twenty out of thirty-three) biochemical reactions were altered with respect to control after biofield energy treatment. About 39.39% out of thirty-three biochemicals, such as acetamide, arginine, citrate, colistin, nitrofurantoin, glucose, indole, nitrate, oxidation-fermentation/glucose, oxidase, penicillin, tartrate, and urea did not show any change in all the groups after biofield treatment as compared to the control (Table 3).

Previously, many organisms under the genus of Providencia have been called as P. rettgeri based on urea hydrolysis biochemical reaction pattern while based on additional fermentation process the organism was reclassified as P. stuartii [20].

3.3. Identification of Organism by Biotype Number

The species (*P. rettgeri*) was identified and distinguished based on morphological characters and biotyping. Biotype is defined as a group of individuals with same genotype. Biotype number of specific strain was evaluated after interpreting the results of the biochemical reactions and led to the particular strain identification. In the present study, biotyping was performed using an automated system, and results showed a significant change in biotype number in all the treated groups as compared to the control. After biofield energy treatment, an alteration of biotype numbers were observed in Gr. IIA on day 10 (40640644; *Providencia rettgeri*), in Gr. IIB; Study I on day 162 (40041544; *Proteus mirabilis*), in Gr. IIB; Study II on day 5 (40041544; *Proteus mirabilis*), on day 10 (40061544; *Proteus mirabilis*) and on day 15 (40041544; *Proteus mirabilis*) as compared to the control (77765376; *Providencia rettgeri*) (Table 4). Brenner *et al.* had proposed the transfer of genus from *Providencia* to *Proteus* [21]. In this experiment, the biochemicals adonitol and inositol showed positive (+) reactions in control as well as in Gr. IIA that supports the identifiable genus *Providencia*, while negative (-) reactions in rest of the treated groups indicated the genus *Proteus* (Table 4). The results were well supported by literature data [22]. Although both genus *i.e. Providencia* and *Proteus* possess same tribe *Proteeae* but they have diverse characteristics [23]. Biofield energy treatment may be responsible for alteration in microorganism at enzymatic and/or genetic level, which may act on receptor protein and that could lead to show different phenotypic characteristics [24].

Table 4. *Effect of biofield treatment on biotype number of Providencia rettgeri.*

Feature	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 162)	Gr. IIB (Study II; Day 162)		
				Day 5	Day 10	Day 15
Biotype	77765376	40640644	40041544	40041544	40061544	40041544
Organism Identification	*P. rettgeri*	*P. rettgeri*	*Proteus mirabilis*	*Proteus mirabilis*	*Proteus mirabilis*	*Proteus mirabilis*

Gr.: Group

Biofield treatment might induce significant changes in lyophilized as well as revived strain of *P. rettgeri* and significantly improved the antimicrobials susceptibility pattern, MIC. It also altered the biochemical reactions which ultimately change the biotype number with new microorganism. As a result, the microbe that was resistance/intermediate/inducible β-lactamase to a particular antimicrobial in control sample now converted into susceptible in the treated cells of *P. rettgeri* predominately after biofield energy treatment. Due to microbial resistance to a single and/or multiple drugs, the invention of an effective antimicrobial therapy for the human-wellness is urgently required. So far our group had reported many scientific evidence regarding the effects on Mr. Trivedi's biofield energy treatment on ATCC and multidrug resistant strains [10, 11]. Based on these results, it is envisaged that biofield energy

treatment has the ability to alter the sensitivity pattern of antimicrobials and a positive scope to be an alternative integrative medicine approach than the existing antimicrobial therapy in near future.

4. Conclusions

In conclusion, the antimicrobial susceptibility pattern and the MIC values showed the significant 78.57% (out of twenty-eight) and 65.63% (out of thirty-three) alteration, respectively of tested antimicrobials as compared to the control strain of *P. rettgeri*. Moreover, about 71.43% antimicrobials sensitivity and 56.25% MIC values of tested antimicrobials were improved after biofield energy treatment to the strain of *P. rettgeri*. Besides, the biochemical reactions pattern showed the significant 60.61% alteration as compared to the control. Moreover, the biotype numbers of biofield treated strain of *P. rettgeri* were also changed in all the treated groups as compared to the control. Based on the changed biotype numbers after biofield treatment, new species was identified as *Proteus mirabilis* in all the treated groups except *P. rettgeri* in Gr. IIA on day 10 as compared to the control. Thus, Mr. Trivedi's unique biofield energy treatment could be applied as an alternative therapeutic approach against antimicrobials to improve the antibiogram profile against microbes. Based on these results, it seems that biofield treatment could be used as an alternate of existing drug therapy in near future.

Abbreviations

NIH/NCCAM: National Institute of Health/National Center for Complementary and Alternative Medicine**;** ATCC: American Type Culture Collection; NBPC 30: Negative Breakpoint Combo 30; MIC: Minimum Inhibitory Concentration

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References

[1] Holt JG (1994) Bergey's manual of determinative bacteriology. (9thedn), Williams & Wilkins, Baltimore, MD.

[2] Armbruster CE, Smith SN, Yep A, Mobley HL (2014) Increased incidence of urolithiasis and bacteremia during *Proteus mirabilis* and *Providencia stuartii* coinfection due to synergistic induction of urease activity. J Infect Dis 209: 1524-1532.

[3] Manos J, Belas R (2006) The genera *Proteus*, *Providencia*, and *Morganella*. Prokaryotes 6: 245-269.

[4] Obayes HS, GAbd F (2013) Pathogenesis of *Providencia rettgeri* in mice. Journal of Babylon University/Pure and Applied Sciences 21: 2785-2800.

[5] Yoh M, Matsuyama J, Ohnishi M, Takagi K, Miyagi H, Mori K, et al. (2005) Importance of *Providencia* species as a major cause of travellers' diarrhoea. J Med Microbiol 54: 1077-1082.

[6] O'Hara CM, Brenner FW, Miller JM (2000) Classification, identification, and clinical significance of *Proteus*, *Providencia* and *Morganella*. Clin Micro Rev 13: 534-546.

[7] Matsuura M, Nakazawa H, Inoue M, Mitsuhashi S (1980) Purification and biochemical properties of beta-lactamase produced by *Proteus rettgeri*. Antimicrob Agents Chemother 18: 687-690.

[8] Koithan M (2009) Introducing complementary and alternative therapies. J Nurse Pract 5: 18-20.

[9] Prakash S, Chowdhury AR, Gupta A (2015) Monitoring the human health by measuring the biofield "aura": An overview. IJAER 10: 27637-27641.

[10] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Phenotypic and biotypic characterization of *Klebsiella oxytoca*: An impact of biofield treatment. J Microb Biochem Technol 7: 203-206.

[11] Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) Antimicrobial sensitivity pattern of *Pseudomonas fluorescens* after biofield treatment. J Infect Dis Ther 3: 222.

[12] Patil SA, Nayak GB, Barve SS, Tembe RP, Khan RR (2012) Impact of biofield treatment on growth and anatomical characteristics of *Pogostemon cablin* (Benth.). Biotechnology 11: 154-162.

[13] Nayak G, Altekar N (2015) Effect of biofield treatment on plant growth and adaptation. J Environ Health Sci 1: 1-9.

[14] Trivedi MK, Tallapragada RR (2008) A transcendental to changing metal powder characteristics. Met Powder Rep 63: 22-28, 31.

[15] Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O (2015) Studies of the atomic and crystalline characteristics of ceramic oxide nano powders after bio field treatment. Ind Eng Manage 4: 161.

[16] Fader RC, Weaver E, Fossett R, Toyras M, Vanderlaan J, et al. (2013) Multilaboratory study of the biomic automated well-reading instrument versus MicroScan WalkAway for reading MicroScan antimicrobial susceptibility and identification panels. J Clin Microbiol 51: 1548-1554.

[17] Penner JL, Preston MA (1980) Differences among *Providencia* species in their *in vitro* susceptibilities to five antibiotics. Antincrob Agents Chemother 18: 868-871.

[18] Stock I, Wiedemann B (1998) Natural antibiotic susceptibility of *Providencia stuartii*, *P. rettgeri*, *P. alcalifaciens*, and *P. rustigianii* strains. J Med Microbiol 47: 629-642.

[19] Mino Y, Kitano S, Morimoto S, Ogihara T (1997) Urinary bacteria in elderly patients with urinary incontinence and low levels of daily activity. Nihon Ronen Igakkai Zasshi 34: 1004-1008.

[20] Farmer JJ 3rd, Hickman FW, Brenner DJ, Schreiber M, Rickenbach DG, (1977) Unusual *Enterobacteriaceae*: "*Proteus rettgeri*" that "change" into *Providencia stuartii*. J Clin Microbiol 6: 373-378.

[21] Brenner DJ, Farmer III JJ, Fanning GR, Steigerwalt AG, Klykken P, et al. (1978) Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species. Int J Syst Bacteriol 28: 269-282.

[22] Brenner Don J, Krieg Noel R, Staley James R (2005) Manual® of Systematic Bacteriology: The *Proteobacteria*, Part B: The Gammaproteobacteria George Garrity, Springer Science & Business Media.

[23] Senior BW (1997) Media and tests identification of bacteria to simplify the recognition and members of the *Proteeae*. J Med Microbiol 46: 39-44.

[24] Lindstrom E, Mild KH, Lundgren E (1998) Analysis of the T cell activation signaling pathway during ELF magnetic field exposure, $p56^{lck}$ and $[Ca^{2+}]_i$-measurements. Bioeletrochem Bioenerg 46: 129-137.