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Phenotyping and 16S rDNA Analysis after Biofield Treatment on *Citrobacter braakii*: A Urinary Pathogen

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Abstract

Citrobacter braakii (*C. braakii*) is widespread in nature, mainly found in human urinary tract. The current study was attempted to investigate the effect of Mr. Trivedi's biofield treatment on *C. braakii* in lyophilized as well as revived state for antimicrobial susceptibility pattern, biochemical characteristics, and biotype number. Lyophilized vial of ATCC strain of *C. braakii* was divided into two parts, Group (Gr.) I: control and Gr. II: treated. Gr. II was further subdivided into two parts, Gr. IIA and Gr. IIB. Gr. IIA was analysed on day 10 while Gr. IIB was stored and analysed on day 159 (Study I). After retreatment on day 159, the sample (Study II) was divided into three separate tubes. First, second and third tube was analysed on day 5, 10 and 15, respectively. All experimental parameters were studied using automated MicroScan Walk-Away® system. The 16S rDNA sequencing of lyophilized treated sample was carried out to correlate the phylogenetic relationship of *C. braakii* with other bacterial species. The antimicrobial susceptibility and minimum inhibitory concentration showed 39.29% and 15.63% alteration respectively in treated cells of *C. braakii* as compared to control. Tetracycline showed improved sensitivity pattern, *i.e.,* from resistant to susceptible after biofield treatment, with support of decreased MIC value (>8 to ≤ 4 µg/mL) by two-fold in all the treated samples as compared to the control. Biochemical reactions also showed significant (42.42%) alteration in the treated samples with respect to the control. Biotype numbers with species were substantially changed in Gr. IIA (53131052, *Citrobacter freundii* complex) on day 10 and in Gr. IIB, Study I (53111052; *Citrobacter amalonaticus*) on day 159 as compared to the control (77365776; *Citrobacter braakii*). Moreover, biotype numbers with species were substantially changed in Gr. IIB, Study II after retreatment on day 5 (53111042, *Citrobacter amalonaticus*) and (53131052; *Citrobacter freundii* complex) on day 10 and 15 as compared to the control. 16S rDNA analysis showed that the identified microbe as *Citrobacter freundii* (GenBank Accession Number: DQ517285) with 95% identity. The nearest homolog genus-species of *C. braakii* was found to be *Citrobacter werkmanii* (Accession No. AF025373). The results suggested that biofield treatment has a significant impact on *C. braakii* in lyophilized as well as revived state.

Keywords: *Citrobacter braakii*; Antimicrobial susceptibility; Biofield treatment; Biochemical reaction; Biotype; 16S rDNA analysis; Gram-negative bacteria; Enterobacteriaceae

Abbreviations: MDR: Multi-Drug Resistant; ATCC: American Type Culture Collection; NBPC 30: Negative Breakpoint Combo 30; MIC: Minimum Inhibitory Concentration; OTUs: Operational Taxonomic Units; NCBI: National Center for Biotechnology Information; MEGA: Molecular Evolutionary Genetics Analysis; PCR: Polymerase Chain Reaction; RDP: Ribosomal Database Project; HBMEC: Human Brain Microvascular Endothelial Cells

Introduction

Citrobacter braakii (*C. braakii*) is a genus of Gram-negative, straight, facultative anaerobic and motile bacilli bacterium widely distributed in water, soil, and food in the environment. It is also commonly found in urinary, intestinal, and respiratory tract of human and animals, belongs to *Enterobacteriaceae* family. It has been associated with various nosocomial and community acquired infections in humans [1]. Arens et al. reported about 11 genetically distinct species within the genus Citrobacter [2]. The main clinical manifestations have been reported due to nosocomial infections of *Citrobacter* species such as bacteremias [3], endocarditis [4], urinary tract infections [1], neonatal meningitis [5], pneumonia [6] and brain abscess [7]. Based on literature it has been demonstrated that 0.8% of Gram-negative infections caused by *Citrobacter* spp. [8]. In hospital settings, about 3-6% *Citrobacter* spp. causes nosocomial infection among all Enterobacteriaceae family [9]. Although it has low virulence property responsible for host cell invasion instead of it invade blood brain barrier (BBB) of human brain microvascular endothelial cells (HBMEC) and causes meningitis. Moreover, due to overproduction of chromosomal β-lactamase enzyme leads to antimicrobials resistance [10]. Aminoglycosides, fluoroquinolones, carbapenems, new oral cephems and many third and fourth-generation cephems, such as cefepime and cefpirome, are the drugs of choice to treat *C. braakii* associated infections but it possess high level of resistance against penicillin and other antibiotics [11,12]. Therefore, an alternative strategy is needed to alter the antimicrobial sensitivity profile against *C. braakii* strain. In recent years, biofield treatment was proved to be an alternative method which has impact on various properties of living and non-living materials in a cost effective manner. It is already demonstrated that energy can neither be created nor be destroyed but it can be transferred through various processes such as thermal, chemical, kinetic, nuclear, etc. [13-15]. Similarly, electrical current exists inside the human body in the form of vibratory energy particles like ions, protons, and electrons and they generate magnetic field in the human body [16,17]. Afterward, Harold Saxton Burr had

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performed the detailed studies on the correlation of electric current with physiological process and concluded that every single process in the human body had an electrical significance [18]. Recently, it was discovered that all electrical process happening in body have strong relationship with magnetic field as mentioned by Ampere's law, which states that the moving charge produces magnetic fields in surrounding space [19,20]. Thus, the human body emits the electromagnetic waves in form of bio-photons, which surround 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. According to Rivera-Ruiz et al., it was reported that electrocardiography has been extensively used to measure the biofield of human body [21]. Thus, human has the ability to harness the energy from environment or universe and can transmit into any living or nonliving object(s) around the Globe. The objects always receive the energy and responding into useful way that is called biofield energy and the process is known as biofield treatment. Mr. 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 [22-24], improved the overall productivity of crops [25,26], altered characteristics features of microbes [27-29] and improved growth and anatomical characteristics of medicinal plants [30,31].

Due to the clinical significance of this organism and literature on biofield treatment, the present work was undertaken to evaluate the impact of biofield treatment on *C. braakii* in relation to antimicrobials susceptibility and biotyping based on various biochemical characters followed by 16S rDNA sequencing analysis.

Materials and Methods

C. braakii, American Type Culture Collection (ATCC 43162) strain was procured from MicroBioLogics, Inc., USA and 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 Negative Breakpoint Combo 30 (NBPC 30) panel with respect to control group (Gr.). The 16S rDNA sequencing study was carried out using ultrapure genomic DNA prep kit; Cat KT 83 (Bangalore Genei, India).

Experimental design

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

Group I: ATCC strain was revived from lyophilized state and considered as control. No treatment was given and 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 *C. braakii* in lyophilized state were assigned to the Mr. Trivedi's unique biofield treatment (first treatment). Gr. IIB sample was stored in lyophilized state for 159 days at -70°C. 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 159 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 -70°C and the revived culture was again provided to Mr. Trivedi's biofield treatment (re-treatment) on day 159. After biofield retreatment, the sample was sub-cultured into three separate tubes on 3 different days (Day 0, Day 5 and Day 10) and analysed keeping the main treated tube aside. Each sample was analyzed after 5 days of its sub-culturing.

Biofield treatment strategy

The lyophilized (Gr. IIA) sample of *C. braakii* was subjected to Mr. Trivedi's biofield treatment (first treatment) followed by retreatment after storing for 159 days in revived state (Gr. IIB, Study II). In details, the treatment groups in sealed pack were handed over to Mr. Trivedi for biofield treatment under laboratory conditions. Mr. Trivedi provided the treatment through his energy transmission process to the treated groups without touching the samples. After first treatment, the analysis of Gr. IIA lyophilized sample was done on day 10 for antimicrobial sensitivity along with minimum inhibitory concentration (MIC), biochemical reactions with biotype number and 16S rDNA analysis as per the standard protocol. While handing over these cultures to Mr. Trivedi for retreatment purposes, optimum precautions were taken to avoid contamination.

Antimicrobial susceptibility test

Investigation of antimicrobial susceptibility of *C. braakii* was carried out with the help of automated instrument, MicroScan Walk-Away® using NBPC 30 panel. The panel can be stored at 2 to 25°C for analysis. The panel was allowed to equilibrate to room temperature prior to 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 *C. braakii* was 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 was performed using the RENOK® system with inoculators-D (B1013-4). 25 mL of standardized inoculum suspension was poured in to inoculum tray. The detailed experimental procedure and conditions were followed as per the manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, R: Resistant; and I: Intermediate) and MIC values were determined by observing the lowest antimicrobial concentration showing inhibition of growth [32].

Biochemical reaction studies

Biochemical reactions of *C. braakii* 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. The detailed experimental procedures and conditions were followed as per the manufacturer's instructions [32].

Identification of organism by biotype number

The biotype number of *C. braakii* was determined on MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data [32].

Amplification and gene sequencing of 16S rDNA

Genomic DNA was isolated from *C. braakii* cells (Gr. IIA, sample coded

as 4A) using genomic purification kit, according to the manufacturer instructions. 16S rDNA gene (~ 1.5 kb) fragment was amplified with the help of high-fidelity polymerase chain reaction (PCR) using universal primers; forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer (3'-ACGGTCATACCTTGTTACGACTT-5'). Amplified products were subjected to gel electrophoresis in 1.0% agarose gel, stained with ethidium bromide and visualized under UV light in a gel documentation unit (BioRad Laboratories, USA). The PCR amplified fragment was purified from the agarose gel using a DNA gel extraction kit. Sequencing of amplified product was done on commercial basis from Bangalore Genei, India. The 16S rDNA sequences obtained were aligned and compared with the sequences stored in GenBank database available from National Center for Biotechnology Information (NCBI) using the algorithm BLASTn program. Multiple sequence alignment/phylogenetic tree were established using MEGA3.1 molecular software [33].

Results and Discussion

Antimicrobial susceptibility test

The results of *C. braakii* susceptibility pattern and MIC values of tested antimicrobials after biofield treatment are presented in Table 1 and 2 respectively. The data were analyzed and compared with respect to control (Gr. I). Antimicrobial susceptibility assay was carried out using twenty-eight antimicrobials. Overall, the treated cells of *C. braakii* showed 39.29% alteration in antimicrobial sensitivity pattern as compared to control. The sensitivity pattern of tetracycline was changed from resistance (R) to susceptible (S) and simultaneously decreased MIC value by two folds (>8 to ≤ 4 µg/mL) in all the treated groups as compared to control (Gr. I). This improvement with respect to resistant pattern and MIC value could be due to biofield treatment. The effect was observed throughout the experiment. So, it may assume that the effect of biofield treatment was sustainable. Moreover, the antibiogram pattern of certain antimicrobials *viz.* aztreonam, cefotaxime, cefotetan, ceftazidime, ceftriaxone, cefuroxime, piperacillin/tazobactam, piperacillin and ticarcillin/k-clavulanate were changed from inducible β-lactamase (IB) to susceptible in lyophilized treated (first treatment) Gr. IIB, Study I on day 159 as well as in revived treated (second treatment) Gr. IIB, Study II on day 5 as compared to untreated sample (Gr. I). The microbe *C. braakii* has the ability to produce chromosomal β-lactamases. Thus, the overproduction of this enzymes lead to resistance in most of penem and cephems ring containing antimicrobials [34].

However, after biofield treatment the above mentioned nine antimicrobials were converted from IB to completely susceptible in lyophilized treated (first treatment) Gr. IIB, Study I on day 159 as well as in revived treated (second treatment) Gr. IIB, Study II on day 5 as compared to control sample. However, the susceptibility pattern of these nine antimicrobials did not show any alteration in rest of the treated samples as compared to control. This alteration could be due to exertion of biofield energy to the treated samples at enzymatic

S. No.	Antimicrobial	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
					Day 5	Day 10	Day 15
1.	Amikacin	S	S	S	S	S	S
2.	Amoxicillin/k-clavulanate	I	I	R	R	R	R
3.	Ampicillin/sulbactam	I	I	I	I	I	I
4.	Ampicillin	R	R	R	R	R	R
5.	Aztreonam	IB	IB	S	S	IB	IB
6.	Cefazolin	R	R	R	R	R	R
7.	Cefepime	S	S	S	S	S	S
8.	Cefotaxime	IB	IB	S	S	IB	IB
9.	Cefotetan	IB	IB	S	S	IB	IB
10.	Cefoxitin	R	R	R	R	R	R
11.	Ceftazidime	IB	IB	S	S	IB	IB
12.	Ceftriaxone	IB	IB	S	S	IB	IB
13.	Cefuroxime	IB	IB	S	S	IB	IB
14.	Cephalothin	R	R	R	R	R	R
15.	Chloramphenicol	S	S	S	S	S	S
16.	Ciprofloxacin	S	S	S	S	S	S
17.	Gatifloxacin	S	S	S	S	S	S
18.	Gentamicin	S	S	S	S	S	S
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	S	S	S	S
23.	Piperacillin/tazobactam	IB	IB	S	S	IB	IB
24.	Piperacillin	IB	IB	S	S	IB	IB
25.	Tetracycline	R	S	S	S	S	S
26.	Ticarcillin/k-clavulanate	IB	IB	S	S	IB	IB
27.	Tobramycin	S	S	S	S	S	S
28.	Trimethoprim /sulphamethoxazole	S	S	S	S	S	S

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

Table 1: Antibiogram of *Citrobacter braakii*: effect of biofield treatment on antimicrobial susceptibility.

levels and that may ceased or reduced formation of β-lactamase from those antimicrobials. As a consequence, these antimicrobials became completely susceptible to *C. braakii* even after two times biofield treatment as compared to control. Beside this, the MIC values of these nine antimicrobials did not show any alteration in all the treated samples while MIC value of cefuroxime was altered by two-fold (8 µg/mL) in Gr IIA on day 10 as compared to the control. The antimicrobial sensitivity pattern and MIC value of amoxicillin/k-clavulanate were changed from I to R and (16/8 to >16/8 µg/mL) respectively in Gr. IIB, Study I on day 159 (first-time biofield treatment) and in Study II on day 5, 10 and 15 after retreatment as compared to control. The MIC value of ESBL-a Scrn was slightly altered in Gr. II, Study II on day 10 after retreatment on day 159 as compared to the control. The MIC value of nitrofurantoin was decreased by two-fold (>64 to ≤ 32 µg/mL) in all the treated samples as compared to untreated sample (Table 2). Overall, 15.63% MIC values of antimicrobials were altered out of thirty-two antimicrobials as compared to control. Rest of antimicrobials did not show any alteration in terms of antibiogram and MIC in all the treated samples as compared to the control.

Biochemical reactions studies

Study of biochemical reactions can be utilized to identify the enzymatic and metabolic characteristic feature of microbes. Microorganisms can be categorically differentiated based on their utilization of specific biochemicals as nutrients during the process of metabolism or enzymatic reactions. Data obtained from biochemical reactions studies for differentiation of *C. braakii* are illustrated in Table 3. Biochemical indole (IND) was changed from negative (-) to positive (+) reaction in all the treated samples as compared to control (Gr. I). The key characteristics of *C. braakii* were positive reactions of ornithine decarboxylase (ORN) production and utilization of malonate (MAL) in control sample of *C. braakii*. The control data were supported with literature [35]. The biochemicals such as adonitol (ADO), colistin (CL4), esculin hydrolysis (ESC), nitrofurantoin (FD64), lysine (LYS), malonate (MAL), raffinose (RAF), sucrose (SUC), tryptophan deaminase (TDA), urea (URE), and Voges-Proskauer (VP) were changed from positive (+) to negative (-) reactions in all the treated samples as compared to control. Moreover, biochemical reaction of H_2S was converted from positive (+) to negative reaction in Gr. IIB, study I on day 159 after first treatment and in Gr. IIB, study II on day 5 after retreatment with Mr. Trivedi's biofield treatment as compared to control. Galactosidase was converted from positive (+) to negative (-) reaction in Gr. IIB, Study II on day 5 after retreated the sample on day 159 while remained same, i.e., positive (+) in all the others groups as compared to control. Overall,

S. No.	Antimicrobial	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
					Day 5	Day 10	Day 15
1.	Amikacin	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16
2.	Amoxicillin/k-clavulanate	16/8	16/8	>16/8	>16/8	>16/8	>16/8
3.	Ampicillin/sulbactam	16/8	16/8	16/8	16/8	16/8	16/8
4.	Ampicillin	>16	>16	>16	>16	>16	>16
5.	Aztreonam	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
6.	Cefazolin	>16	>16	>16	>16	>16	>16
7.	Cefepime	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
8.	Cefotaxime	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
9.	Cefotetan	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16
10.	Cefoxitin	>16	>16	>16	>16	>16	>16
11.	Ceftazidime	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
12.	Ceftriaxone	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
13.	Cefuroxime	≤ 4	8	≤ 4	≤ 4	≤ 4	≤ 4
14.	Cephalothin	>16	>16	>16	>16	>16	>16
15.	Chloramphenicol	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
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	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
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	≤ 2	≤ 2	≤ 2	≤ 2
25.	Nitrofurantoin	>64	≤ 32	≤ 32	≤ 32	≤ 32	≤ 32
26.	Norfloxacin	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
27.	Piperacillin/tazobactam	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16
28.	Piperacillin	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16
29.	Tetracycline	>8	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
30	Ticarcillin/k-clavulanate	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16
31.	Tobramycin	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
32.	Trimethoprim/sulphamethoxazole	≤ 2/38	≤ 2/38	≤ 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 a and b screen

Table 2: Effect of biofield treatment on *Citrobacter braakii* to minimum inhibitory concentration (MIC) value of tested antimicrobials.

S. No.	Code	Biochemical	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
						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.	H_2S	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 *Citrobacter braakii* to the biochemical reactions pattern.

biochemical reactions showed significant (42.42%) alteration in the treated groups with respect to control. Rest of the biochemicals did not show any alteration of biochemical reactions in all the treated groups as compared to control (Table 3).

Identification of organism by biotype number

The species (*C. braakii*) were identified based on a 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 an automated system, and results showed a significant change in biotype number (53131052) in Gr. IIA (on day 10) after first-time biofield treatment with identification of new species (*Citrobacter freundii* complex) as compared to control Gr. I (77365776; *C. braakii*). The term *Citrobacter freundii* complex have eight species out of 11 identified genomospecies under the genus Citrobacter *viz. Citrobacter jeundii*, *Citrobacter youngae*, *Citrobacter braakii*, *Citrobacter werkmanii*, *Citrobacter sedlakii* and three unnamed *Citrobacter* species [2]. After that, these three genomospecies were named as *Citrobacter rodentium*, *Citrobacter gillenii*, and *Citrobacter murliniae* [35].

Moreover, the biotype numbers were also changed on day 159 in Gr. IIB, Study I (53111052; *Citrobacter amalonaticus*) as well as in Gr. IIB, Study II after retreatment on day 5 (53111042; *Citrobacter amalonaticus*), day 10 (53131052; *Citrobacter freundii* complex), and on day 15 (53131052; *Citrobacter freundii* complex) as compared to the control (Table 4). These changes of biotype numbers may be due to alteration of several biochemical reactions under the influence of biofield treatment in the lyophilized state. Furthermore it is further assumed that the changes of biotype numbers could be due to first-time biofield treatment and sustained effects upto day 159.

16S rDNA genotyping

The bacteria that are poorly differentiated by conventional methods needs molecular analysis method like 16S rDNA sequence [36]. This molecular based technique is suitable tool for identification of most of bacteria on their genus and/or species level by comparison with databases in the public domain. Because, most of bacteria have small ribosomal subunit with their species-specific variability [37]. The 16S rDNA sequence was determined in *C. braakii* on Gr. IIA sample. The alignment and comparison of the consensus gene sequences were performed with the sequences stored in GenBank database

Feature	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
				Day 5	Day 10	Day 15
Biotype	77365776 (Very rare biotype)	53131052	53111052	53111042	53131052	53131052
Organism Identification	*Citrobacter braakii*	*Citrobacter freundii* complex	*Citrobacter amalonaticus*	*Citrobacter amalonaticus*	*Citrobacter freundii* complex	*Citrobacter freundii* complex

Gr.: Group

Table 4: Effect of biofield treatment on *Citrobacter braakii* assessment of biotype number.

Alignment View	AN	Alignment Result	Sequence Description
	4A	0.96	Sample studied
	DQ517285	0.95	*Citrobacter freundii* strain BRN1
	DQ444289	0.98	*Citrobacter freundii* strain 6
	AF025365	0.99	*Citrobacter freundii*
	AY567708	0.98	*Candidatus cuticobacterium* kirbyi
	DQ294285	0.97	*Citrobacter freundii* strain 7
	AF025368	1.00	*Citrobacter braakii*
	DQ294286	0.96	*Citrobacter freundii* strain 8
	AB210978	0.98	*Citrobacter freundii* strain: SSCT56
	AF025373	0.98	*Citrobacter werkmanii*
	DQ517286	0.95	*Citrobacter freundii* strain BRN2

AN: GenBank Accession Number

Table 5: The closest sequences of *Citrobacter braakii* from sequence alignment using NCBI GenBank and ribosomal database project (RDP).

available from NCBI using the algorithm BLASTn program. Based on nucleotide homology and phylogenetic analysis the microbe (Sample 4A) was detected as *Citrobacter freundii* (GenBank Accession Number: DQ517285) with 95% identity. The nearest homolog genus-species of C. *braakii* was found to be *Citrobacter werkmanii* (Accession No. AF025373). Some other close homologs of C. *braakii* were found from the alignment results as shown in Table 5. The distance matrix based on nucleotide sequence homology data are presented in Table 6. Phylogenetic tree was established using BLAST-Webpage (NCBI). According to Table 6, ten different related bacterial species of *C. braakii* were selected as Operational Taxonomic Units (OTUs) in order to investigate the phylogenetic relationship of *C. braakii*. There were 1498 base nucleotides of 16S rDNA gene sequences, which were analyzed and multiple alignments were constructed using ClustalW in MEGA3.1. The numbers of base substitutions per site from pairwise distance analysis between sequences are shown in Table 5. All results were based on the pairwise analysis of 11 sequences. According to the data in Table 6, the lowest value of genetic distance from *C. freundii* strain BRN1 was 0.004 base substitutions per site. This value is due to the distance between *C. braakii* and *C. freundii*. All pairwise distance analysis was carried out using the p-distance method in MEGA3.1. The proportion of remarked distance, sometimes also called p-distance and showed as the number of nucleotide distances site. Values in Table 5 are programmed into Figure 1 with optimal bootstrap consensus tree. In the phylogram, there were eleven OTUs. The results suggested that *C. braakii* was closely related to the *C. freundii* with 95% similarity and the lowest genetic distance 0.004 base substitutions per site.

Biofield treatment might be responsible for alteration in microorganism at genetic level and/or enzymatic level, which may act on receptor protein. While altering receptor protein, ligand-receptor/

Distance Matrix												
AN		1	2	3	4	5	6	7	8	9	10	11
AF025365	1	—	0.997	0.999	0.995	0.998	0.995	0.991	0.995	0.994	0.996	0.994
AF025368	2	0.003	—	0.996	0.994	0.995	0.992	0.989	0.994	0.993	0.995	0.991
DQ444289	3	0.001	0.004	—	0.995	0.998	0.993	0.991	0.994	0.993	0.996	0.994
DQ294286	4	0.005	0.006	0.005	—	0.995	0.990	0.988	0.993	0.989	0.995	0.991
AY567708	5	0.002	0.005	0.002	0.005	—	0.993	0.991	0.993	0.992	0.995	0.993
AB210978	6	0.005	0.008	0.007	0.010	0.007	—	0.986	0.990	0.988	0.991	0.988
DQ517285	7	0.009	0.011	0.009	0.012	0.009	0.014	—	0.989	0.991	0.989	0.996
AF025373	8	0.005	0.006	0.006	0.007	0.007	0.010	0.011	—	0.992	0.994	0.989
DQ517286	9	0.006	0.007	0.007	0.011	0.008	0.012	0.009	0.008	—	0.990	0.988
DQ294285	10	0.004	0.005	0.004	0.005	0.005	0.009	0.011	0.006	0.010	—	0.991
4A	11	0.006	0.009	0.006	0.009	0.007	0.012	0.004	0.011	0.012	0.009	—

AN: GenBank Accession Number

Table 6: Distance matrix of *Citrobacter braakii* sample based on nucleotide sequence homology (using kimura-2 parameter).

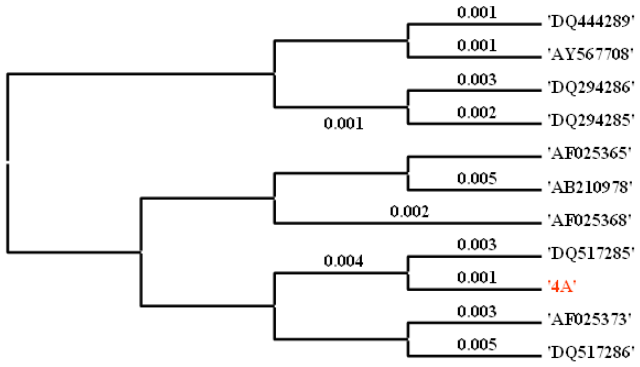

Figure 1: Phylogenetic tree of the partial 16S rDNA gene sequencing of *Citrobacter braakii* using MEGA 3.1 software using neighbor joining method. Numbers represent GenBank accession number.

protein interactions may alter that could lead to show different phenotypic characteristics [38]. Biofield treatment might induce significant changes in lyophilized strain of *C. braakii* and alter antimicrobials susceptibility pattern, MIC values, biochemical reactions, which ultimately change the biotype number of microorganism. As a result, the microbe that was intermediate/resistant to a particular antimicrobial in control sample now converted into susceptible in treated cells of *C. braakii* predominately after biofield treatment. In this experiment, the main objective was to see the impact of Mr. Trivedi's biofield treatment on an opportunistic hospital acquired pathogen of *C. braakii* in *in vitro*. Based on above findings the antimicrobials those are resistance/inducible β-lactamase producing now converted into absolutely susceptible after biofield treatment. So far our group had been published many research articles regrading short-term effects on biofield treatment on ATCC and multidrug resistant (MDR) strains [27-29]. This is the first report exploring the sustained effects of Trivedi's biofield treatment on microorganism *i.e. C. braakii*. Based on these results, it is expected that biofield treatment has the scope to be an alternative approach than the existing antimicrobial therapy in near future.

Conclusion

In conclusion, the antimicrobial susceptibility pattern and MIC values showed 39.29% and 15.63% alteration, respectively of tested antimicrobials as compared to the control strain of *C. braakii*. The biochemical reactions pattern showed significant (42.42%) alteration as compared to control. Moreover, the biotype numbers of biofield treated strain of *C. braakii* were also changed in all the treated groups as compared to control. Based on changed biotype numbers after biofield treatment, new species were identified as *Citrobacter freundii* complex and *Citrobacter amalonaticus* in treated cells with respect to control Gr. I (77365776; *C. braakii*). Thus, Mr. Trivedi's unique biofield treatment could be applied as an alternative therapeutic approach against antimicrobials resistance. Molecular based 16S rDNA analysis showed that the treated lyophilized sample in this experiment was *C. braakii* and was converted to *Citrobacter freundii* (GenBank Accession Number: DQ517285) after biofield treatment. However, the nearest homolog genus-species was found to be *Citrobacter werkmanii* (Accession No. AF025373). Based on these results, it seems that biofield treatment could be used as alternate of existing drug therapy in future.

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References

1. Metri BC, Jyothi P, Peerapur BV (2013) Antibiotic resistance in *Citrobacter* spp. isolated from urinary tract infection. Urol Ann 5: 312-313.
2. Arens S, Verbist L (1997) Differentiation and susceptibility of Citrobacter isolates from patients in a university hospital. Clin Microbiol Infect 3: 53-57.
3. Drelichman V, Band JD (1985) Bacteremias due to *Citrobacter diversus* and *Citrobacter freundii*. Incidence, risk factors, and clinical outcome. Arch Intern Med 145: 1808-1810.
4. MacCulloch D, Menzies R, Cornere BM (1977) Endocarditis due to *Citrobacter diversus* developing resistance to cephalothin. N Z Med J 85: 182-183.
5. Williams WW, Mariano J, Spurrier M, Donnell HD Jr, Breckenridge RL Jr, et al. (1984) Nosocomial meningitis due to *Citrobacter diversus* in neonates: New aspects of the epidemiology. J Infect Dis 150: 229-235.
6. Mateos Rodríguez F, Pérez Moro E, Atienza Morales MP, Beato Pérez JL (2000) [Community-acquired bacteremic pneumonia due to *Citrobacter diversus*]. An Med Interna 17: 165-166.
7. Curless RG (1980) Neonatal intracranial abscess: Two cases caused by Citrobacter and a literature review. Ann Neurol 8: 269-272.
8. Jones RN, Jenkins SG, Hoban DJ, Pfaller MA, Ramphal R (2000) In vitro efficacy of six cephalosporins tested against Enterobacteriaceae isolated at 38 North American medical centers participating in the SENTRY antimicrobial surveillance program, 1997 - 1998. Int J Antimicrorb Agents 15: 111-118.
9. Lavigne JP, Defez C, Bouziges N, Mahamat A, Sotto A (2007) Clinical and molecular epidemiology of multidrug-resistant *Citrobacter* spp. infections in a French university hospital. Eur J Clin Microbiol Infect Dis 26: 439-441.
10. Badger JL, Stins MF, Kim KS (1999) Citrobacter freundii invades and replicates in human brain microvascular endothelial cells. Infect Immun 67: 4208-4215.
11. Samonis G, Anaissie E, Elting L, Bodey GP (1991) Review of *Citrobacter bacteremia* in cancer patients over a sixteen-year period. Eur J Clin Microbiol Infect Dis 10: 479-485.
12. Shih CC, Chen YC, Chang SC, Luh KT, Hsieh WC (1996) Bacteremia due to *Citrobacter* species: significance of primary intra-abdominal infection. Clin Infect Dis 23: 543-549.
13. Becker RO, Selden G (1998) The body electric: Electromagnetism and the foundation of life. (1stedn), William Morrow and Company. New York City,
14. Barnes RB (1963) Thermography of the human body. Science 140: 870-877.
15. Born M (1971) The Born-Einstein Letters. (1stedn), Walker and Company, New York.
16. Planck M (1903) Treatise on thermodynamics. (3rdedn), English translated by Alexander OGG, Longmans, Green, London (UK).
17. Einstein A (1905) Does the inertia of a body depend upon its energy-content? Ann Phys 18: 639-641.
18. Burr HS (1957) Bibliography of Harold Saxton Burr. Yale J Biol Med 30: 163-167.
19. Hammerschlag R, Jain S, Baldwin AL, Gronowicz G, Lutgendorf SK, et al. (2012) Biofield research: A roundtable discussion of scientific and methodological issues. J Altern Complement Med 18: 1081-1086.
20. Movaffaghi Z, Farsi M (2009) Biofield therapies: Biophysical basis and biological regulations? Complement Ther Clin Pract 15: 35-37.
21. Rivera-Ruiz M, Cajavilca C, Varon J (2008) Einthoven's string galvanometer: The first electrocardiograph. Tex Heart Inst J 35: 174-178.
22. 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.
23. Dabhade VV, Tallapragada RR, Trivedi MK (2009) Effect of external energy on atomic, crystalline and powder characteristics of antimony and bismuth powders. Bull Mater Sci 32: 471-479.
24. Trivedi MK, Tallapragada RM (2009) Effect of super consciousness external energy on atomic, crystalline and powder characteristics of carbon allotrope powders. Mater Res Innov 13: 473-480.
25. Sances F, Flora E, Patil S, Spence A, Shinde V (2013) Impact of biofield treatment on ginseng and organic blueberry yield. Agrivita J Agric Sci 35: 22-29.

26. Lenssen AW (2013) Biofield and fungicide seed treatment influences on soybean productivity, seed quality and weed community. Agricultural Journal 83: 138-143.

27. 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.

28. Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) An effect of biofield treatment on multidrug-resistant *Burkholderia cepacia*: A multihost pathogen. J Trop Dis 3: 167.

29. 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.

30. 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.

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

32. 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.

33. Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform 5: 150-163.

34. Pepperell C, Kus JV, Gardam MA, Humar A, Burrows LL, et al. (2002) Low-virulence *Citrobacter* species encode resistance to multiple antimicrobials. Antimicrob Agents Chemother 46: 3555-3560.

35. Brenner DJ, O'hara CM, Grimont PA, Janda JM, Falsen E, et al. (1999) Biochemical identification of *Citrobacter* species defined by DNA hybridization and description of *Citrobacter gillenii* sp. nov. (Formerly Citrobacter genomospecies 10) and *Citrobacter murliniae* sp. nov. (Formerly Citrobacter genomospecies 11). J Clin Microbiol 37: 2619-2624.

36. Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, et al. (2000) 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J Clin Microbiol 38: 3623-3630.

37. Vandamme P, Pot B, Gillis M, de Vos P, Kersters K, et al. (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60: 407-438.

38. Lindstrom E, Mild KH, Lundgren E (1998) Analysis of the T cell activation signaling pathway during ELF magnetic field exposure, p56lck and [Ca2+] i-measurements. Bioeletrochem Bioenerg 46: 129-137.