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Antibiogram and Genotypic Analysis using 16S rDNA after Biofield Treatment on *Morganella morganii*

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Abstract

Morganella morganii (*M. morganii*) is one of the important nosocomial pathogen associated with the urinary tract infections and bacteremia. The aim of this study was to evaluate the effect of Mr. Trivedi's biofield energy treatment on *M. morganii* in the lyophilized as well as revived state for antimicrobial susceptibility pattern, biochemical characteristics, biotype number and genotype. *M. morganii* cells were procured from MicroBioLogics Inc., USA in sealed packs bearing the American Type Culture Collection (ATCC 25829) number and stored according to the recommended storage protocols until needed for experiments. *M. morganii* strain was divided into two groups, Group (Gr.) I: control and Gr. II: treated. Gr. II was further subdivided into two groups, Gr. IIA and Gr. IIB. Gr. IIA was analyzed on day 10, while Gr. IIB was stored and analyzed on day 142 (Study I). After retreatment on day 142, the sample (Study II) was divided into three separate tubes. First, second and third tube was further analyzed on day 5, 10 and 15 respectively. All experimental parameters were studied using the automated MicroScan Walk-Away® system. The 16S rDNA sequencing of lyophilized treated sample was carried out to correlate the phylogenetic relationship of *M. morganii* with other bacterial species. Antimicrobial susceptibility results showed 32.14% alterations, while minimum inhibitory concentration results showed 18.75% alterations of the tested antimicrobials. Biochemical study also showed altered positive reactions in nitrofurantoin and indole with respect to control. Biotype study showed alteration in Gr. IIB, study II, on day 15 (4005 1446) as compared to the control (4004 1446). 16S rDNA sequencing analysis showed similar results with the identified microbe as *M. morganii* (GenBank accession number: AB210972) having 80% identity of the gene sequencing data. Total 1507 base nucleotide of 16S rDNA gene sequences were analyzed by multiple alignments, while nearest homolog genus-species of *M. morganii* was found as *Providencia rettgeri* (accession number: AM040492). These results suggested that biofield treatment has a significant impact on *M. morganii* in lyophilized as well as revived state.

Keywords: *Morganella morganii*; Antimicrobial susceptibility; Biofield energy; Biochemical reaction; Biotype; 16S rDNA analysis

Abbreviations: NCCAM: National Center for Complementary and Alternative Medicine; 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

Introduction

Morganella species are the clinically characterized in the tribe Proteeae [1]. *Morganella* species infections are less frequent in healthy individuals. *Morganella morganii* (*M. morganii*) is Gram-negative and facultative anaerobic bacterium of the family Enterobacteriaceae. It exists as commensal within the intestinal tract of humans, mammals, and reptiles as a normal flora [2]. Originally, it was reported to be as common cause of summer diarrhoea but later on, clinical infections associated with urinary tract, skin and soft tissue and hepatobiliary tract were reported [3]. They are motile, non-lactose fermenting, and have the capacity for urease production and show the presence of phenylalanine deaminase. Morganella associated infections are the fifth leading cause of UTIs in nursing home patients [4]. According to Warren et al. catheter-associated bacteriuria due to Morganella in long term care facilities has been reported [5]. Most of the infectious cases in microbiology laboratory were associated with urine, wounds, and from a variety of body fluids or tube drainage [6]. *M. morganii* bacteremia entry has involved hepatobiliary tract, as 64% cases are related to intra-abdominal infections [7]. *Morganella* species show resistance against β-lactam antibiotics, usually due to the presence of chromosomally encoded β-lactamases belonging to the *AmpC* β-lactamase family. Most of the β-lactamase enzymes are inducible in nature only on exposure to antibiotics [8]. Continuous use of antibiotics leads to the high-level expression of a resistant isolate of *M. morganii* against second or third generation's antibiotics [9]. Therefore, an alternative strategy is needed to alter the antimicrobial sensitivity profile against Morganella strain. Multidrug therapy and some alternate approach are required to treat the associated infections. Due to the several side effects alternate and complementary therapy approach are the best treatment strategies. Among various complementary and alternate treatment approach, biofield treatment may be one of the approach to alter the antimicrobial sensitivity.

Biofield has been defined as "energy fields that purportedly surround and penetrate the human body". Biofield treatment refers to a group of energy therapy that affects people's health and well-being by interacting with their biofield. According to physics, "energy" defines as the capacity to do work, and overcome resistance, while "field" refers to the force which can cause action at a distance. As a basic law in physics,

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when electrical signals fluctuate with time, the magnetic field generates in the surroundings. After detection of biomagnetism in laboratories, many researchers hypothesized that the flow of bioelectricity in human bodies possibly the reason of biomagnetic fields. On the other hand, specific environmental frequencies are absorbed by the biomolecules, results in alterations in the movements of component parts [10]. Biofield therapies practice involved the alteration in consciousness states in mind. These healing modalities or touch therapies were mainly used to reduce the pain, anxiety, and promote health [11]. Healing treatment suggests the mechanism upon modulating patient-environmental energy fields, as its main treatment approach is through bioelectromagnetics and biophysical fields that form the major role in cellular structure and function of the human body [12]. Thus, the human body emits the electromagnetic waves in the form of bio-photons and moving electrically charged particles (ions, cell, molecule, etc.), which surround the body. Thus, human has the ability to harness the energy from the 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 the useful way that is called biofield energy and the process is known as biofield treatment. Mr. Trivedi's unique biofield energy is also known as The Trivedi effect®, which has been reported to alter the structural, physical and thermal properties of several metals and ceramics in material science research [13-15], improved the overall productivity of crops [16,17], altered characteristics features of microbes [18-20] and improved growth and anatomical characteristics of medicinal plants [21,22].

Due to the significant impact of biofield treatment, and clinical importance of *M. morganii*, the study was designed to evaluate the impact of Mr. Trivedi's biofield energy treatment on *M. morganii* in relation to study the phenotypic and genotypic characters of organism using 16S rDNA sequencing analysis.

Materials and Methods

M. morganii, American Type Culture Collection (ATCC 25829) 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).

Inoculum preparation

The turbidity standard technique using direct inoculation of revived and lyophilized *M. morganii* was used. Using a sterile wooden applicator stick or bacteriological loop, the surfaces of 4-5 large or 5-10 small morphologically similar cultures were touched for well-isolated colonies from an 18-24 hour non-inhibitory agar plate. Further, colonies were emulsified in 3 mL of inoculum water (autoclaved deionized water) to an equivalent of a 0.5 McFarland barium sulfate turbidity standard. 100 µL of the standardized suspension was pipetted into 25 mL of inoculum water using pluronic and inverted 8-10 times.

Experimental design

The impact of biofield treatment on tested bacterium *M. morganii* was evaluated in two groups.

Group I: ATCC strain in lyophilized state was 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 *M. morganii* in lyophilized state were assigned to the Mr. Trivedi's unique biofield treatment. Gr. IIB sample was stored in lyophilized state for 142 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 142 days, the sample was revived and tested for 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 provided to Mr. Trivedi's biofield treatment (re-treatment) on day 142. After biofield retreatment, the sample was sub-cultured into three separate tubes on three different days (Day 0, Day 5 and Day 10) and analyzed keeping the main treated tube aside. Each sample was analyzed after 5 days of its sub-culturing.

Biofield energy treatment strategy

The lyophilized sample of *M. morganii* was subjected to Mr. Trivedi's biofield energy treatment (first treatment) which was analyzed on day 10 (Gr. IIA), followed by retreatment after storing for 142 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 [20].

Antimicrobial susceptibility test

Investigation of antimicrobial susceptibility of *M. morganii* was carried out with the help of automated instrument, MicroScan Walk-Away® using NBPC 30 panel. The panel can be stored at 2 to -25ºC 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 *M. morganii* was pipetted into 25 mL of inoculum water using pluronic, inverted 8 to 10 times and inoculated, rehydrated, and then subjected to incubation for 16 hours at 35ºC. Rehydration and inoculation were performed using the RENOK® system with inoculators-D (B1013-4). 25 mL of standardized inoculum suspension was poured into inoculum tray. The detailed experimental procedure and conditions were followed as per the manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, R: Resistant; I: Intermediate, and IB; Inducible β-lactamases) and MIC values were determined by observing the lowest antimicrobial concentration showing inhibition of growth [23].

Biochemical reaction studies

Biochemical reactions of *M. morganii* 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 [23].

Identification of organism by biotype number

The biotype number of *M. morganii* was determined on MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data [23].

Amplification and gene sequencing of 16S rDNA

Genomic DNA was isolated from *M. morganii* cells (Gr. IIA, sample coded as 5A) 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') [24]. 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 [25].

Results and Discussion

Antimicrobial susceptibility test

Antimicrobial sensitivity result are summarized in Table 1 and compared with respect to control group (Gr. I). Aztreonam showed significant alterations in all the experimental groups after first biofield treatment and retreatment. Control group showed sensitivity of aztreonam as Inducible β-lactamases (IB), but after biofield treatment in lyophilized state, sensitivity was improved as susceptible (S) in Gr. IIA, day 10. Further, sensitivity was altered as Intermediate (I) in Gr. IIB, study I, day 142 with respect to control (Gr. I). After retreatment in revived state, sensitivity of aztreonam was altered as Resistant (R) in Gr. IIB, study II, on day 5, 10, and 15 as compared to control (Gr. I). Cefotaxime, cefotetan, cefoxitin, ceftriaxone, piperacillin, piperacillin/tazobactam, and ticarcillin/k-clavulanate showed altered sensitivity from IB to S in Gr. IIA (day 10), Gr. IIB (study I, day 142), and Gr. IIB (study II, day 5 and 10) as compared to control (Gr. I), while again showed similar sensitivity as IB in Gr. IIB, study II (day 15). Ceftazidime showed altered sensitivity, i.e., from IB to S in Gr. IIA (day 10), and Gr. IIB, study II (day 142), while it was changed to Resistant (R) and Intermediate (I) in Gr. IIB, study II on day 5 and 10 respectively, as

S. No.	Antimicrobial	Gr. I	Gr. IIA	Gr. IIB, Study I	Gr. IIB, Study II		
		Control	Day 10	Day 142	Day 5	Day 10	Day 15
1	Amikacin	S	S	S	S	S	S
2	Amoxicillin/k-clavulanate	R	R	R	R	R	R
3	Ampicillin/sulbactam	R	R	R	R	R	R
4	Ampicillin	R	R	R	R	R	R
5	Aztreonam	IB	S	I	R	R	R
6	Cefazolin	R	R	R	R	R	R
7	Cefepime	S	S	S	S	S	S
8	Cefotaxime	IB	S	S	S	S	IB
9	Cefotetan	IB	S	S	S	S	IB
10	Cefoxitin	IB	S	S	S	S	IB
11	Ceftazidime	IB	S	S	R	I	IB
12	Ceftriaxone	IB	S	S	S	S	IB
13	Cefuroxime	R	R	R	R	R	R
14	Cephalothin	R	R	R	R	R	R
15	Chloramphenicol	I	I	I	I	I	I
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	S	S	S	S	IB
24	Piperacillin	IB	S	S	R	R	IB
25	Tetracycline	R	R	R	R	R	R
26	Ticarcillin/k-clavulanate	IB	S	S	S	S	IB
27	Tobramycin	S	S	S	S	S	S
28	Trimethoprim/ Sulfamethoxazole	S	S	S	S	S	S

R: Resistant; I: Intermediate; S: Susceptible; IB: Inducible β-lactamases; Gr: Group; **Antimicrobial susceptibility pattern in control and treated groups were evaluated using automated MicroScan Walk-Away® system using NBPC30 panel.**

Table 1: Effect of biofield treatment on antimicrobial susceptibility pattern of *Morganella morganii*.

compared to control (Gr. I). Piperacillin showed altered sensitivity *i.e.* from IB to S in Gr. IIA (day 10), Gr. IIB, study II (day 142), while it was changed to resistant (R) in Gr. IIB, study II on day 5 and 10, as compared to control (Gr. I). Overall, biofield treatment on *M. morganii* showed alteration in 32.14% of tested antimicrobials out of twenty-eight results in antimicrobial sensitivity pattern with respect to control. Rest of the antimicrobials did not show any change in sensitivity pattern after biofield treatment.

Biofield treatment on *M. morganii* showed altered MIC values of tested antimicrobials and results are reported in Table 2. Aztreonam showed two folds change in MIC value as (>16 µg/mL) in Gr. IIB, study II, day 5, 10, and 15, while 16 µg/mL in Gr. IIB, study I (day 142) as compared to control (≤ 8 µg/mL, Gr. I). Ceftazidime showed altered MIC, i.e., from ≤ 8 µg/mL to >16 µg/mL (Gr. IIB, study II, day 5) and 16 µg/mL (Gr. IIB, study II, day 10) as compared to control (Gr. I). ESBL-a Scrn showed decrease MIC value (≤ 4 µg/mL) in Gr. IIB, study I, day 142 after biofield treatment, while ESBL-b Scrn showed altered MIC values (>1 µg/mL) in Gr. IIB, study II, day 5 as compared to control (Gr. I). Nitrofurantoin showed altered MIC value (>64 µg/mL) in Gr. IIA, day 10, while piperacillin showed altered MIC values after re-treatment (>64 µg/mL) in Gr. IIB, study II, day 5 and 10 with respect to control (Gr. I). Overall data suggest that 18.75% alteration out of total thirty-two tested antimicrobials in MIC values after biofield treatment. Rest of the tested antimicrobials did not show any change in MIC value after biofield treatment with respect to control.

Proteeae are normal fecal flora and have been disturbed by antibiotic therapy, *M. morganii* belongs to the tribe Proteeae of family Enterobacteriaceae. Different reports of infections associated with urinary tract infections, skin and soft tissue infections, meningitis and bacteremia often with fatal consequences [26,27]. The suggested treatment is based on symptoms while initiate treatment starts with an extended-spectrum cephalosporin or penicillin combined with an aminoglycoside. The preferred β-lactam antibiotics include aztreonam, piperacillin, cefepime, ceftazidime, and piperacillin-tazobactam. The microbe *M. morganii* has the ability to produce *AmpC*-lactamase. Thus, the overproduction of these enzymes leads to resistance in most of preferred β-lactam antibiotics via *ampR* gene [28-30]. Biofield treatment on *M. morganii* leads to change the sensitivity of antimicrobials such as cefotaxime, cefotetan, cefoxitin, ceftazidime, and ceftriaxone from inducible β-lactamases into susceptible. This improved sensitivity

S. No.	Antimicrobial	Gr. I	Gr. IIA	Gr. IIB, Study I	Gr. IIB, Study II		
		Control	Day 10	Day 142	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	16	>16	>16	>16
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	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
11	Ceftazidime	≤ 8	≤ 8	≤ 8	>16	16	≤ 8
12	Ceftriaxone	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8	≤ 8
13	Cefuroxime	>16	>16	>16	>16	>16	>16
14	Cephalothin	>16	>16	>16	>16	>16	>16
15	Chloramphenicol	16	16	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	≤ 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	>64	64	64	64	64
26	Norfloxacin	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
27	Piperacillin/tazobactam	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16
28	Piperacillin	≤ 16	≤ 16	≤ 16	> 64	> 64	≤ 16
29	Tetracycline	>8	>8	>8	>8	>8	>8
30	Ticarcillin/k-clavulanate	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16	≤ 16
31	Tobramycin	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4
32	Trimethoprim/ Sulfamethoxazole	≤ 2/38	≤ 2/38	≤ 2/38	≤ 2/38	≤ 2/38	≤ 2/38

MIC values are presented in µg/mL; Gr: Group; ESBL: Suspected extended-spectrum β-lactamases a, b screen; MIC values in control and treated groups were evaluated using automated MicroScan Walk-Away® system using NBPC30 panel.

Table 2: Minimum Inhibitory Concentration (MIC) of *Morganella morganii* for tested antimicrobials.

with respect to control could be due to biofield energy treatment that might alter the resistance mechanism and the effect was sustained till 142 days with respect to control. Overall, biofield treatment has impact on lyophilized as well as revived state with respect to antimicrobial sensitivity.

Biochemical reactions studies

Biochemical reactions can determine the nutritional and metabolic capabilities of a bacterial isolate, which is the most common approach for determining the genus and species of an organism. The combination of reactions are available and used to establish the enzymatic capabilities of microbes as well as the ability to grow and survive in the presence of certain inhibitors used in various biochemical reactions [31]. Data obtained from biochemical reactions studies for differentiation of *M. morganii* after biofield treatment is illustrated in Table 3. Basic characteristic of biochemical reactions of *M. morganii* are reported as negative reactions in adonitol, arabinose, hydrogen sulfide, malonate, melibiose, sorbitol, sucrose, and Voges-Proskauer, while positive reactions of glucose, ornithine, and urea [27]. Experimental control results were well supported with literature data. Biofield treatment showed altered biochemical reaction of Indole (IND), *i.e.*, negative (-) to positive (+) reaction in Gr. IIB, study II, day 15 as compared to control (Gr. I). However, nitrofurantoin (FD64) showed negative (-) to positive (+) reaction after biofield treatment in the lyophilized state in Gr. IIA, day 10 as compared to control (Gr. I). Rest of the tested biochemicals did not show any change in reaction pattern after biofield treatment (Table 3). Our group has recently reported the significant effect of biofield treatment on *Burkholderia cepacia* and *Pseudomonas fluorescens* with altered characteristic biochemical reactions [19,20].

Identification of organism by biotype number

M. morganii was further 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 change in biotype

S. No.	Code	Biochemical	Type of Response					
			Gr. I	Gr. IIA	Gr. IIB; Study I	Gr. IIB; Study II		
			Control	Day 10	Day 142	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	+	+	+	+	+	+
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; Biochemical reactions in control and treated groups were evaluated using automated MicroScan Walk-Away® system using NBPC30 panel.

Table 3: Effect of biofield treatment on biochemical reactions of *Morganella morganii*.

number (4005 1446) in Gr. IIB, study II (on day 15) as compared to control Gr. I (4004 1446). Alteration in species was reported in Gr. IIB, study I and Gr. IIB, study II (day 5) group with maximum probability identified as *Proteus mirabilis* after biofield treatment. This change of biotype number may be due to alteration of some biochemical reactions under the influence of biofield treatment.

16S rDNA genotyping

In order to confirm the PCR-based identification result, 16S rDNA sequence analysis was performed in biofield treated *M. morganii* strain. It includes PCR assays and DNA amplification using standard forward and reverse 16S universal primers. 16S rDNA amplification protocol has been commonly used as a taxonomic "gold standard" in the identification and determining the phylogenies of bacterial species [32]. 16S rDNA sequence molecular analysis was used to differentiate and find the closely related microorganism of treated microbe, better identification tool than conventional method [33]. 16S rDNA sequencing analysis can be correlated with results of biotype number based on altered biochemical reactions.

The alignment and assessment of the gene sequences data were performed by comparing with the sequences available in GenBank database of NCBI, using the algorithm BLASTn program. The phylogenetic tree was constituted using BLAST-Webpage (NCBI). Ten closely related bacterial species and *Morganella morganii* were considered as Operational Taxonomic Units (OTUs) in order to investigate the phylogenetic relationship of *M. morganii* among other ten related species (Figure 1). Total 1507 base nucleotide of 16S rDNA gene sequences were analyzed by multiple alignments using ClustalW of MEGA3.1 program. Based on the phylogenetic tree and 16S rDNA sequencing, the nearest homolog species was found to be *Providencia rettgeri* (Accession No: AM040492). Other closely related homologs of *M. morganii* can be found from the sequence alignment as shown in Table 4. Distance matrix between the 16S-rDNA sequences of 11 pathogens was analyzed based on nucleotide sequence homology using Kimura-2 Parameter. According to the data in Table 5, the lowest value of genetic distance from sample 5A was 0.003 base substitutions per site. Total 11 sequences of base substitutions per site from pairwise distance analysis were shown in Table 5. Based on nucleotides homology and phylogenetic analysis the microbe (sample 5A) was detected to be *Morganella morganii* (GenBank Accession Number: AB210972) with 80% identity of gene sequencing data.

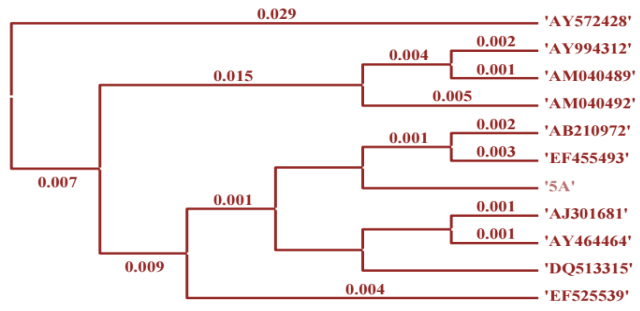

Figure 1: Phylogenetic tree of the partial 16S rDNA gene sequencing of *Morganella morganii* using MEGA 3.1 software using neighbor joining method. Numbers represent GenBank accession number.

Biofield therapies are very popular in biomedical heath care systems as holistic medicine, which are considered significant by National Center for Complementary and Alternative Medicine (NCCAM) [34]. NCCAM places the biofield therapy (putative energy fields) in the subcategory of energy therapies as one of the five complementary medicine domains [35]. Biofield treatment on pathogenic microbes has been reported to alter the susceptibility pattern of antimicrobials. Data suggest that biofield energy might alter the microorganism at genetic and/or enzymatic level, which could be responsible for the change in sensitivity of antimicrobials and biochemical reactions. Based on above findings the antimicrobials those are resistance/inducible β-lactamase now converted into susceptible after biofield treatment. Antimicrobial interactions might alter at ligand-receptor level/protein level that leads to show different phenotypic characteristics [36]. Experimental design and results suggest that alterations might occur even after storage of sample in -70ºC for 142 days. It suggests that Mr. Trivedi's unique biofield treatment has the ability to alter the antimicrobial sensitivity in treated *M. morganii* even in the lyophilized storage condition for a long duration. 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.

Alignment View	AN	Alignment results	Sequence description
	5A	0.80	Sample studied
	DQ513315	0.80	*Morganella morganii* strain VAR-06-2076
	AY464464	0.78	*Morganella morganii*
	AJ301681	0.79	*Morganella morganii* strain CIPA231T
	EF525539	0.78	*Morganella morganii* strain RP-42
	EF455493	0.79	*M. morganii* subsp. *morganii* strain 06-136
	AB210972	0.80	*Morganella morganii* strain: SSCT63
	AY994312	0.93	*Providencia alcalifaciens*
	AM040489	0.94	*Providencia rustigianii* type strain DSM 4541
	AM040492	0.90	*Providencia rettgeri* type strain DSM 4542
	AY572428	0.95	*Hafnia alvei*

AN: GenBank Accession Number; Alignment results and sequence description has been obtained from the blast results of GenBank database of National Center for Biotechnology Information (NCBI) using the algorithm BLASTn program.

Table 4: The closest sequences of *Morganella morganii* from sequence alignment using NCBI GenBank and Ribosomal Database Project (RDP).

Distance Matrix													
AN		1	2	3	4	5	6	7	8	9	10	11	12
AJ301681	1	—	0.996	0.999	0.989	0.994	0.967	0.999	0.968	0.953	0.969	0.995	0.999
AB210972	2	0.004	—	0.997	0.987	0.993	0.965	0.997	0.966	0.950	0.966	0.995	0.997
AY464464	3	0.001	0.004	—	0.990	0.995	0.968	1	0.969	0.953	0.970	0.996	0.999
AY043168	4	0.011	0.013	0.011	—	0.988	0.964	0.990	0.964	0.949	0.965	0.987	0.990
EF525539	5	0.006	0.007	0.005	0.012	—	0.966	0.995	0.967	0.951	0.968	0.992	0.994
AY994312	6	0.033	0.035	0.032	0.036	0.034	—	0.968	0.997	0.944	0.990	0.964	0.968
DQ513315	7	0.001	0.004	0.000	0.011	0.005	0.032	—	0.969	0.953	0.970	0.996	0.999
AM040489	8	0.032	0.034	0.031	0.036	0.034	0.003	0.031	—	0.946	0.990	0.965	0.969
AY572428	9	0.047	0.050	0.047	0.051	0.049	0.056	0.047	0.054	—	0.940	0.950	0.953
AM040492	10	0.031	0.034	0.030	0.035	0.032	0.011	0.030	0.011	0.060	—	0.966	0.969
EF455493	11	0.005	0.005	0.004	0.013	0.008	0.036	0.004	0.035	0.050	0.034	—	0.997
5A	12	0.001	0.003	0.001	0.010	0.006	0.032	0.001	0.031	0.047	0.031	0.004	—

AN: GenBank Accession Number; Nucleotide similarity is denoted above diagonal, while distance as below diagonal identities between the studied sample '5A' and ten other closest homologs microbe. Total 1507 base nucleotide of 16S rDNA gene sequences were analyzed by multiple alignments using ClustalW program. Pairwise distance (lower left) and number of nucleotide difference (upper-right) for 16S forward and reverse primer was presented using Kimura-2 Parameters.

Table 5: Distance matrix of biofield treated *Morganella morganii* based on nucleotide sequence homology (Using Kimura-2 Parameter).

Conclusion

In conclusion, Mr. Trivedi's biofield treatment on *M. morganii* showed the altered antimicrobial sensitivity of 32.14% tested antimicrobials. The MIC values of 18.75% tested antimicrobials were altered after biofield energy treatment in *M. morganii*. Characteristics biochemical test of *M. morganii* such as indole and nitrofurantoin reactions were altered followed by a change in the biotype number (4005 1446, Gr. IIB, study II) as compared to control (4004 1446). Thus, Mr. Trivedi's unique biofield energy treatment could be applied to alter the antimicrobials resistance pattern. Molecular based 16S rDNA analysis showed that the identifiable sample in this experiment was detected as *M. morganii* (GenBank Accession Number: AB210972) with 80% identity of the gene sequencing data after biofield treatment. Based on the phylogenetic tree and 16S rDNA sequencing, the nearest homolog species was found as *Providencia rettgeri* (Accession No. AM040492). Based on these results, it seems that Mr. Trivedi's biofield energy treatment could be used as an alternate of the existing drug therapy in future.

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References

1. Braunstein H, Tomasulo M (1978) Identification of *Proteus morganii* and distinction from other *Proteus* species. Am J Clin Pathol 70: 905-908.
2. Falagas ME, Kavvadia PK, Mantadakis E, Kofteridis DP, Bliziotis IA, et al. (2006) *Morganella morganii* infections in a general tertiary hospital. Infection 34: 315-321.
3. Lin TY, Chan MC, Yang YS, Lee Y, Yeh KM, et al. (2015) Clinical manifestations and prognostic factors of *Morganella morganii* bacteremia. Eur J Clin Microbiol Infect Dis 34: 231-236.
4. Nicolle LE, Strausbaugh LJ, Garibaldi RA (1996) Infections and antibiotic resistance in nursing homes. Clin Microbiol Rev 9: 1-17.
5. Warren JW, Tenney JH, Hoopes JM, Muncie HL, Anthony WC (1982) A prospective microbiologic study of bacteriuria in patients with chronic indwelling urethral catheters. J Infect Dis 146: 719-723.
6. McDermott C, Mylotte JM (1984) *Morganella morganii*: Epidemiology of bacteremic disease. Infect Control 5: 131-137.
7. Kim BN, Kim NJ, Kim MN, Kim YS, Woo JH, et al. (2003) Bacteraemia due to tribe Proteeae: A review of 132 cases during a decade (1991-2000). Scand J Infect Dis 35: 98-103.
8. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39: 1211-1233.
9. Stock I, Wiedemann B (1998) Identification and natural antibiotic susceptibility of *Morganella morganii*. Diagn Microbiol Infect Dis 30: 153-165.
10. Movaffaghi Z, Farsi M (2009) Biofield therapies: Biophysical basis and biological regulations? Complement Ther Clin Pract 15: 35-37.
11. Cahil M (1998) Nurses handbook of complementary and alternative therapies. Springhouse, PA: Springhouse Corporation.
12. Schwartz GE, Simon WL, Carmona R (2007) The energy healing experiments: Science reveals our natural power to heal. (1stedn), Atria Books.
13. 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.
14. Trivedi MK, Patil S, Nayak G, Jana S, Latiyal O (2015) Influence of biofield treatment on physical, structural and spectral properties of boron nitride. J Material Sci Eng 4: 181.
15. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyal O (2015) Evaluation of biofield treatment on physical, atomic and structural characteristics of manganese (II, III) oxide. J Material Sci Eng 4: 177.
16. 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.
17. Lenssen AW (2013) Biofield and fungicide seed treatment influences on soybean productivity, seed quality and weed community. Agricultural Journal 83: 138-143.
18. 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.
19. 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.
20. 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.
21. 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.
22. Nayak G, Altekar N (2015) Effect of biofield treatment on plant growth and adaptation. J Environ Health Sci 1: 1-9.

23. Fader RC, Weaver E, Fossett R, Toyras M, Vanderlaan J, et al. (2013) Multi-laboratory 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.

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

25. Alm EW, Oerther DB, Larsen N, Stahl DA, Raskin L (1996) The oligonucleotide probe database. Appl Environ Microbiol 62: 3557-3559.

26. Williams EW, Hawkey PM, Penner JL, Senior BW, Barton LJ (1983) Serious nosocomial infection caused by *Morganella morganii* and *Proteus mirabilis* in a cardiac surgery unit. J Clin Microbiol 18: 5-9.

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

28. Yang YJ, Livermore DM (1988) Chromosomal beta-lactamase expression and resistance to beta-lactam antibiotics in *Proteus vulgaris* and *Morganella morganii*. Antimicrob Agents Chemother 32: 1385-1391.

29. Jacoby GA (2009) AmpC beta-lactamases. Clin Microbiol Rev 22: 161-182.

30. Barnaud G, Arlet G, Verdet C, Gaillot O, Lagrange PH, et al. (1998) *Salmonella enteritidis*: AmpC plasmid-mediated inducible beta-lactamase (DHA-1) with an ampR gene from *Morganella morganii*. Antimicrob Agents Chemother 42: 2352-2358.

31. MacFaddin JF (2000) Biochemical tests for identification of medical bacteria. (3rdedn), Lippincott Williams and Wilkins, Philadelphia, PA.

32. Woese CR (1987) Bacterial evolution. Microbiol Rev 51: 221-271.

33. 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.

34. Koithan M (2009) Introducing Complementary and Alternative Therapies. J Nurse Pract 5: 18-20.

35. http://nccam.nih.gov/health/whatiscam/

36. Hintz KJ, Yount GL, Kadar I, Schwartz G, Hammerschlag R, et al. (2003) Bioenergy definitions and research guidelines. Altern Ther Health Med 9: A13-30.

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