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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 re-treatment 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 (5311052, Citrobacter freundii complex) on day 10 and in Gr. IIB, Study I (5311052, 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 re-treatment 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 cepahems and many third and fourth-generation cephamems, 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
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 Ampère’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 Gencite, 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, cefetan, cefadiazime, 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 microbes 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 level.

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

| S. No. | Antimicrobial                | Gr. I (Control) | Gr. IIA (Day 10) | Gr. IIB (Study I; Day 159) | Gr. IIB (Study II; Day 159) |
|-------|------------------------------|----------------|-----------------|---------------------------|---------------------------|
| 1     | Amikacin                     | S              | S               | S                         | S                         |
| 2     | Amoxicillin/k-clavulinate     | I              | I               | R                         | R                         |
| 3     | Ampicillin/sublactam          | I              | I               | I                         | I                         |
| 4     | Ampicillin                    | R              | R               | R                         | R                         |
| 5     | Aztreonam                     | IB             | IB              | S                         | IB                        |
| 6     | Cefazolin                     | R              | R               | R                         | R                         |
| 7     | Cefepime                      | S              | S               | S                         | S                         |
| 8     | Cefotaxime                    | IB             | IB              | S                         | IB                        |
| 9     | Cefotetan                     | IB             | IB              | S                         | IB                        |
| 10    | Cefoxitin                     | R              | R               | R                         | R                         |
| 11    | Cefazidime                    | IB             | IB              | S                         | IB                        |
| 12    | Ceftriaxone                   | IB             | IB              | S                         | IB                        |
| 13    | Cefuroxime                    | IB             | IB              | S                         | IB                        |
| 14    | Cephalothin                   | R              | R               | R                         | R                         |
| 15    | Chloramphenicol               | S              | S               | S                         | S                         |
| 16    | Ciprofloxacin                 | S              | S               | S                         | S                         |
| 17    | Gatifloxacin                  | S              | S               | S                         | S                         |
| 18    | Gentamicin                    | S              | S               | S                         | S                         |
| 19    | Imipenem                      | S              | S               | S                         | S                         |
| 20    | Levofloxacin                  | S              | S               | S                         | S                         |
| 21    | Meropenem                     | S              | S               | S                         | S                         |
| 22    | Moxifloxacin                  | S              | S               | S                         | S                         |
| 23    | Piperacillin/tazobactam       | IB             | IB              | S                         | IB                        |
| 24    | Piperacillin                  | IB             | IB              | S                         | IB                        |
| 25    | Tetracycline                  | R              | S               | S                         | S                         |
| 26    | Ticarcillin/k-clavulanate     | IB             | IB              | S                         | IB                        |
| 27    | Tobramycin                    | S              | S               | S                         | S                         |
| 28    | Trimethoprim/sulphamethoxazole| S              | S               | S                         | S                         |

R: Resistant; S: Susceptible; I: Intermediate; IB: Inducible β-lactamase; Gr.: Group
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 H₂S 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,
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. Biotyping number then led to the particular organism was evaluated after interpreting the results of the biochemical reactions. The biotype number then to the particular organism identification. In this experiment, biotyping was performed with the sequences stored in GenBank database with their species-specific variability [37]. 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 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. Moreover, the biotype numbers were also changed on day 159 in Gr. IIB, Study I (53110152; *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 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.

| Table 3: Effect of biofield treatment on *Citrobacter braakii* to the biochemical reactions pattern. |
|---|---|---|---|---|---|
| No. | Code | Biochemical | Gr. I (Control) | Gr. IIA (Day 10) | Gr. IIB (Study I; Day 159) | Gr. IIB (Study II; Day 159) |
| 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. | FDB | 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 | Melibiase | + | + | + | + |
| 19. | NIT | Nitrate | + | + | + | + |
| 20. | OFG | 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

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...
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/
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 predominantly 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 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 β-lactamase producing now converted into absolutely susceptible in treated cells of Citrobacter 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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