Investigation of Biofield Treatment on Antimicrobial Susceptibility, Biochemical Reaction Pattern and Biotyping of Enteropathogenic Multidrug-Resistant Escherichia coli Isolates

Mahendra Kumar Trivedi, Alice Branton, Dahryn Trivedi, Gopal Nayak, Harish Shettigar, Mayank Gangwar, Snehasis Jana

To cite this version:

Mahendra Kumar Trivedi, Alice Branton, Dahryn Trivedi, Gopal Nayak, Harish Shettigar, et al.. Investigation of Biofield Treatment on Antimicrobial Susceptibility, Biochemical Reaction Pattern and Biotyping of Enteropathogenic Multidrug-Resistant Escherichia coli Isolates. General Medicine: Open Access, Omics Publishing Group, 2015, S2 (2), https://www.omicsonline.org/open-access/investigation-of-biofield-treatment-on-antimicrobial-susceptibility-biochemical-reaction-pattern-and-biotyping-of-enteropathogenic-multidrugresistant-escherichia-coli-isolates-2327-5146-1000S2-002.php?aid=62301 . 10.4172/2327-5146.1000S2-002 . hal-01445504

HAL Id: hal-01445504

https://hal.archives-ouvertes.fr/hal-01445504

Submitted on 25 Jan 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Investigation of Biofield Treatment on Antimicrobial Susceptibility, Biochemical Reaction Pattern and Biotyping of Enteropathogenic Multidrug-Resistant Escherichia coli Isolates

Mahendra Kumar Trivedi1, Alice Branton1, Dahryn Trivedi1, Gopal Nayak1, Harish Shettigar1, Mayank Gangwar1 and Snehasis Jana2

1Trivedi Global Inc., 10624 S Eastern Avenue Suite A-999, Henderson, NV 89052, USA
2Trivedi Science Research Laboratory Pvt. Ltd., Hall-A, Chinarr Mega Mall, Chinarr Fortune City, Hoshangabad Rd., Bhopal-462026, Madhya Pradesh, India

Corresponding author: Dr. Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Hall-A, Chinara Mega Mall, Chinara Fortune City, Hoshangabad Rd., Bhopal-462026, Madhya Pradesh, India, Tel: +91-755-6660006; E-mail: publication@trivedisrl.com

Rec date: August 01, 2015 Acc date: August 22, 2015 Pub date: August 31, 2015

Abstract

Study background: Multidrug resistant Escherichia coli (MDR E. coli) has become a major health concern, and failure of treatment leads to huge health burden. Aim of the present study was to determine the impact of Mr. Trivedi’s biofield treatment on E. coli.

Methods: Four MDR clinical lab isolates (LSs) of E. coli (LS 8, LS 9, LS 10, and LS 11) were taken and divided into two groups i.e. control and biofield treated. Control and treated samples were identified with respect to its antimicrobial sensitivity assay, biochemical study and biotype number using MicroScan Walk-Away® system. The analysis was done on day 10 after biofield treatment and compared with its respective control group.

Results: Antimicrobial sensitivity assay showed 50% alteration in sensitivity of total tested antimicrobials in treated group of MDR E. coli isolates. MIC results showed the alteration in MIC of about 40.63% antimicrobials out of thirty two tested antimicrobials, after biofield treatment in clinical isolates of E. coli. Ticarclillin/k-clavulanlate showed improved sensitivity (R → I) with decreased MIC in LS 9 as compared to control. A fourfold and twofold decrease in MIC values were reported in case of piperacillin/tazobactam (in LS 9) and chloramphenicol (in LS 8), respectively as compared to respective control. Biochemical study showed a 39.39% alteration in biochemical reactions after treatment among four isolates of E. coli as compared to control. A significant change in biotype numbers were reported in three clinical isolates (i.e. LS 8, LS 9, and LS 11) of MDR E. coli as compared to control. On the basis of changed biotype number (7774 5272) after biofield treatment, organism with maximum probability was identified as Enterobacter aeroerogenes in LS 8 as compared to control, (E. coli; 7711 5012).

Conclusion: Overall results suggest that Mr. Trivedi’s biofield treatment has a significant effect on altering the antimicrobial sensitivity, biochemical reactions and biotype number of MDR isolates of E. coli.

Keywords: Escherichia coli; Biofield treatment; Multidrug-resistant; Antimicrobial susceptibility; Biochemical reaction; Biotyping

Abbreviations:

CLSI: Clinical and Laboratory Standards Institute; MDR: Multidrug-Resistant; MIC: Minimum Inhibitory Concentration; NBPC 30: Negative Breakpoint Combo 30; UTI: Urinary Tract Infection; LS: Clinical Lab Isolates; CAM: Complementary and Alternative Medicine; EBL: Suspected Extended-spectrum β-lactamases; ESBLs: Extended Spectrum β-Lactamases

Introduction

Escherichia coli (E. coli) is a Gram-negative, rod shape, and facultative anaerobic bacteria predominantly found in human colonic flora. Despite the fact about E. coli, its commensal nature and common existence in microflora of animal intestine including man, all the E. coli strains are not harmful; sometimes it causes fatal enteric infections in humans as well as mammals and birds [1]. Pathogenic strains of E. coli cause common intestine infection i.e. diarrhea, extra intestinal infections in humans and animals includes urinary tract infections (UTI), meningitis, and septicemia [2]. Apart from these diseases, pathogenic E. coli may be responsible for causing cystitis and pyelonephritis, a major cause in approximately 80% of 130-175 million human UTIs [3]. Furthermore, E. coli causing extra intestinal infections are the major Gram-negative bacterial pathogens responsible for neonatal meningitis, and ranks second in an overall cause of the disease after group B streptococcus infections [4,5]. During last few years, increasing emergence and wide dissemination of E. coli isolates show resistance to broad-spectrum antimicrobial agents [6]. MDR isolates are the basic cause of failure in treatment modalities, resulting high rate of morbidity and mortality [7]. An emergence of resistance against multiple antimicrobials drugs is a serious threat to public health, and sometimes no available antimicrobials will be effective to treat the infections caused by MDR E. coli [6,8]. Due to dramatically increase in drug resistance against antibiotics, some alternate approach is required to later the sensitivity pattern of antimicrobials. Recently, biofield treatment on pathogenic microorganism is available as an alternative approach to altering the sensitivity pattern of various antimicrobials [9,10].
Biofield as an energy medicine has been included in complementary and alternative medicine (CAM) therapies, and very commonly practiced in US by professional healthcare representative [11]. CAM therapies are very helpful to improve the human wellbeing and health without having any side effects. Bio-electrographic method is non-invasive technology used to measure the human biofield which can evaluate the physical and emotional heath [12]. The energy exists in various forms that can be produced from different sources such as potential, electrical, kinetic, magnetic, and nuclear energy.

The cumulative effect of bio-magnetic and electric field that surrounds the human body is defined as biofield, and the extent of energy associated with biofield is termed as biofield energy. It can be monitored using techniques such as electromyography (EMG), electrocardiography (ECG) and electroencephalogram (EEG) [13]. Mr Mahendra Kumar Trivedi has the ability to harness the energy from environment or universe and can transmit into any living or non-living object(s) around the Universe.

The objects always receive the energy and responding into useful way via biofield energy and the process is known as biofield treatment. Mr Trivedi’s unique biofield treatment is also known as The Trivedi Effect® and it was widely studied in the field of material science [14-16], agricultural science [17-19], and in biotechnology [20]. In microbiology, biofield treatment on pathogenic microbes and MDR isolates had been reported to alter the antimicrobial sensitivity, biochemical reactions and biotype number [10,21,22].

In continuation of outstanding results of biofield treatment and clinical significance of MDR E. coli, present work was designed to evaluate the influence of biofield treatment on MDR isolates of E. coli with respect to its antimicrobials susceptibility, biochemical reactions pattern, and biotyping.

Material and Methods

Bacterial isolates, study design and biofield treatment

MDR clinical lab isolates (i.e. LS 8, LS 9, LS 10 and LS 11) of E. coli were obtained from stored stock cultures in Microbiology Lab, Hinduja Hospital, Mumbai. Each MDR isolate was divided into two groups i.e. control and treatment. 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.

The biofield treated samples were returned in the similar sealed condition and further analyzed on day 10 using the standard protocols. The following parameters like antimicrobial susceptibility, minimum inhibitory concentration (MIC), biochemical reactions, and biotype number were measured in all four MDR E. coli isolates by MicroScan Walk-Away® (Dade Behring Inc., USA) of both control and treated samples. All antimicrobials and biochemicals were procured from Sigma Aldrich.

Inoculum preparation

The turbidity standard technique using direct inoculation of E. coli cell was used in the experiment. Using a sterile wooden applicator stick or bacteriological loop, the surface of 4-5 large or 5-10 small morphologically similar culture was 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.

Evaluation of antimicrobial susceptibility assay

Antimicrobial susceptibility patterns of MDR lab isolates of E. coli were studied using MicroScan Walk-Away® using Negative Break Point Combo (NBPC 30) panel as per the clinical and laboratory standards institute (CLSI) guidelines. The tests carried out on MicroScan were miniaturized of the broth dilution susceptibility test that have been dehydrated. Briefly, the standardized suspension of E. coli were inoculated, rehydrated, and then subjected to incubation for 16 hours at 35°C. The detailed experimental procedures and conditions were followed as per the manufacturer’s instructions. The antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate, R: Resistant, and EBL: Suspected extended-spectrum beta-lactamases) and MIC values were determined by observing the lowest antimicrobial concentration showing growth inhibition [23].

Identification by biochemical study and biotype number

Biochemical studies of each MDR isolates of E. coli were determined by MicroScan Walk-Away® using NBPC 30 panel system in both control and treated groups. The biotype number of each MDR isolates of E. coli in control and treated sample were determined followed by identification of microorganism by MicroScan Walk-Away® processed panel data report with the help of biochemical reaction data [23].

Results and Discussion

Antimicrobial susceptibility study

MDR isolates of E. coli showed altered pattern of antimicrobial sensitivity as compared to its respective control in all the isolates after biofield treatment. Results of antimicrobial sensitivity pattern and MIC values of control and treated MDR isolates of E. coli are summarized in Tables 1 and 2, respectively. Overall, 50% of tested antimicrobials out of thirty two, showed alteration in antimicrobial sensitivity pattern against biofield treated MDR isolates of E. coli. The alterations in sensitivity pattern after biofield treatment were observed as 40.62% in LS 8, 6.25% in LS9, 25% in LS 10, and 6.25% in LS 11 (Figure 1) with respect to control. In this study, very high resistant rates of MDR E. coli isolates against tested antimicrobials such as ampicillin, cefotaxime, ceftriaxone, cefazidime, tetracycline, tobramycin, and aztreonam had been detected (Table 1).

Enterobacteriaceae, such as E. coli and Klebsiella spp., produce different beta-lactamase enzymes, some have activity against penicillin, 2nd, and 3rd generation cephalosporin. However, in recent years, the activity of β-lactamases has been enhanced, as they have the capacity to hydrolyze the extended spectrum cephalosporin, such as cefotaxime, ceftriaxone, cefazidime etc. [24]. Extended spectrum beta-lactamases (ESBLs) are rapidly evolved group of beta-lactamases enzyme, which confer resistance not only against beta-lactam antibiotics, but also for non-penicillin antibiotics [25].
Table 1: Effect of biofield treatment on multidrug resistant lab isolates of *Escherichia coli* to antimicrobial susceptibility. C: Control; T: Treatment; R: Resistant; I: Intermediate; S: Susceptible; LS: Lab Isolate; ESBL-a,b Srcn: Extended-Spectrum-β-Lactamase Screen; -: Not tested; EBL?: Suspected Extended-spectrum β-Lactamases.

| S. No | Antimicrobial          | LS 8   | LS 9   | LS 10  | LS 11  |
|-------|------------------------|--------|--------|--------|--------|
| 1     | Amikacin               | S      | R      | R      | R      | S      | R      | S      |
| 2     | Amoxicillin/k-clavulanate | S    | I      | I      | I      | S      | R      | R      |
| 3     | Ampicillin/sulbactam   | I      | R      | R      | R      | R      | R      | R      |
| 4     | Ampicillin             | R      | R      | R      | R      | R      | R      | R      |
| 5     | Aztreonam              | EBL?   | R      | EBL?   | EBL?   | EBL?   | EBL?   |
| 6     | Cefazolin              | R      | R      | R      | R      | R      | R      | R      |
| 7     | Cefepime               | R      | R      | R      | R      | R      | R      | R      |
| 8     | Cefotaxime             | EBL?   | R      | EBL?   | EBL?   | EBL?   | EBL?   |
| 9     | Cefotetan              | S      | I      | S      | S      | S      | R      | R      |
| 10    | Cefoxitin              | I      | R      | R      | R      | S      | R      | R      |
| 11    | Ceftazidime            | EBL?   | R      | EBL?   | EBL?   | EBL?   | EBL?   |
| 12    | Ceftriaxone            | EBL?   | R      | EBL?   | EBL?   | EBL?   | EBL?   |
| 13    | Cefuroxime             | R      | R      | R      | R      | R      | R      | R      |
| 14    | Cephalothin            | R      | R      | R      | R      | R      | R      | R      |
| 15    | Chloramphenicol        | I      | S      | R      | R      | R      | R      | R      |
| 16    | Ciprofloxacin          | R      | R      | R      | R      | R      | R      | R      |
| 17    | ESBL-a Scn             | EBL?   | -      | EBL?   | EBL?   | EBL?   | EBL?   |
| 18    | ESBL-b Scn             | EBL?   | -      | EBL?   | EBL?   | EBL?   | EBL?   |
| 19    | Gatifloxacin           | R      | R      | R      | R      | I      | R      | R      |
| 20    | Gentamicin             | R      | R      | R      | R      | R      | R      | R      |
| 21    | Imipenem               | S      | S      | S      | S      | S      | S      | I      |
| 22    | Levofloxacin           | R      | R      | R      | R      | R      | R      | R      |
| 23    | Meropenem              | S      | I      | S      | S      | S      | S      | S      |
| 24    | Moxifloxacin           | R      | R      | R      | R      | R      | R      | R      |
| 25    | Nitrofurantoin         | -      | -      | -      | -      | -      | -      | -      |
| 26    | Norfloxacin            | -      | -      | -      | -      | -      | -      | -      |
| 27    | Piperacillin/tazobactam| S      | R      | I      | S      | S      | I      | R      |
| 28    | Piperacillin           | R      | R      | R      | R      | R      | R      | R      |
| 29    | Tetracycline           | R      | R      | R      | R      | R      | R      | R      |
| 30    | Ticarcillin/k-clavulanate| S   | R      | R      | I      | S      | I      | R      |
| 31    | Tobramycin             | R      | R      | R      | R      | R      | R      | R      |
| 32    | Trimethoprim/sulfamethoxazole | R  | R | R | R | S | R | R | R |
### Table 2: Minimum inhibitory concentration (MIC) of multidrug resistant lab isolates of *Escherichia coli*. MIC values are presented in µg/mL; C: Control; T: Treatment; LS: Lab Isolate.

Experimental results of antimicrobial sensitivity assay showed altered sensitivity pattern after biofield treatment in clinical isolates of *E. coli*. Aztreonam, cefotaxime, ceftazidime, and ceftriaxone sensitivity changed from EBL to R, after biofield treatment in LS 8. β-Lactamase

| S No. | Antimicrobial                  | LS 8 |    | LS 9 |    | LS 10 |    | LS 11 |    |
|-------|-------------------------------|------|----|------|----|-------|----|-------|----|
|       |                               | C    | T  | C    | T  | C     | T  | C     | T  |
| 1     | Amikacin                      | ≤16  | >32| >32  | >32| ≤16   | >32| ≤16   | >32|
| 2     | Amoxicillin/k-clavulanate      | 8/4  | 16-Aug| 16-Aug| 8/4| 16-Aug| 8/4| 16-Aug| 8/4|
| 3     | Ampicillin/sulbactam          | 16-Aug| >16/8| >16/8| >16/8| >16/8| >16/8| >16/8| >16/8|
| 4     | Ampicillin                    | >16  | >16| >16  | >16| >16   | >16| >16   | >16|
| 5     | Aztreonam                     | >16  | >16| >16  | >16| >16   | >16| >16   | >16|
| 6     | Cefazolin                     | >16  | >16| >16  | >16| >16   | >16| >16   | >16|
| 7     | Cefepime                      | >16  | >16| >16  | >16| >16   | >16| >16   | >16|
| 8     | Cefotaxime                    | >32  | >32| >32  | >32| >32   | >32| >32   | >32|
| 9     | Cefotetan                     | ≤16  | 32| ≤16  | 32| ≤16   | 32| ≤16   | 32|
| 10    | Cefoxitin                     | 16   | >16| >16  | >16| >16   | >16| >16   | >16|
| 11    | Ceftazidime                   | >16  | >16| >16  | >16| >16   | >16| >16   | >16|
| 12    | Ceftriaxone                   | >32  | >32| >32  | >32| >32   | >32| >32   | >32|
| 13    | Cefuroxime                    | >16  | >16| >16  | >16| >16   | >16| >16   | >16|
| 14    | Cephalothin                   | >16  | >16| >16  | >16| >16   | >16| >16   | >16|
| 15    | Chloramphenicol               | 16   | ≤8| >16  | >16| >16   | >16| >16   | >16|
| 16    | Ciprofloxacin                 | >2   | >2| >2   | >2| >2    | >2| >2    | >2|
| 17    | ESBL-a Scrn                   | >4   | >4| >4   | >4| >4    | >4| >4    | >4|
| 18    | ESBL-b Scrn                   | >1   | >1| >1   | >1| >1    | >1| >1    | >1|
| 19    | Gentamicin                    | >8   | >8| >8   | >8| >8    | >8| >8    | >8|
| 20    | Gentamicin                    | >4   | >4| >4   | >4| >4    | >4| >4    | >4|
| 21    | Imipenem                      | ≤4   | ≤4| ≤4   | ≤4| ≤4    | ≤4| ≤4    | ≤4|
| 22    | Levofloxacin                  | >4   | >4| >4   | >4| >4    | >4| >4    | >4|
| 23    | Meropenem                     | ≤4   | ≥4| ≤4   | ≥4| ≤4    | ≥4| ≤4    | ≥4|
| 24    | Moxifloxacin                  | >4   | >4| >4   | >4| >4    | >4| >4    | >4|
| 25    | Nitrofurantoin                | ≤32  | >64| ≤32  | >64| ≤32   | >64| ≤32   | >64|
| 26    | Norfloxacin                   | >8   | >8| >8   | >8| >8    | >8| >8    | >8|
| 27    | Piperacillin/tazobactam       | ≤16  | >64| ≤16  | >64| ≤16   | >64| ≤16   | >64|
| 28    | Piperacillin                  | >64  | >64| >64  | >64| >64   | >64| >64   | >64|
| 29    | Tetracycline                  | >8   | >8| >8   | >8| >8    | >8| >8    | >8|
| 30    | Ticarcillin/k-clavulanate     | ≤16  | >64| ≤16  | >64| ≤16   | >64| ≤16   | >64|
| 31    | Tobramycin                    | >8   | >8| >8   | >8| >8    | >8| >8    | >8|
| 32    | Trimethoprim/sulfamethoxazole | >2/38| >2/38| >2/38| >2/38| >2/38| >2/38| >2/38| >2/38|
production is very common mechanism of resistance in Enterobacteriaceae family.

However, some pathogenic strains are not able to induce the production of beta-lactamase. Continuous exposure of certain antibiotics, results in enhanced production of AmpC beta-lactamases, termed as induction. Amount of enzymes depends on the time and concentration of antibiotics [24]. Biofield treatment on MDR isolates of E. coli might alter the beta-lactamase genes, which may enhance the production of beta-lactamase enzyme leading to resistant in case of aztreonam, ceftaxime, ceftazidime, and ceftriaxone.

Amikacin, piperacillin/tazobactam, ticarcillin/k-clavulanate sensitivity were altered from S to R, amoxicillin/k-clavulanate, cefotetan, and meropenem were changed from S to I, and ampicillin/sulbactam, cefoxitin changed from I to R, while chloramphenicol sensitivity changed from I to S in LS 8. Sensitivity of ticarcillin/k-clavulanate was improved i.e. from R to I, in biofield treated LS 9 as compared to control. Piperacillin/tazobactam sensitivity altered from I to S as compared to control in LS 9. Amikacin, amoxicillin/k-clavulanate, cefotetan, cefoxitin, and trimethoprim/sulfamethoxazole sensitivity changed from S to R in biofield treatment. Piperacillin/tazobactam, and ticarcillin/k-clavulanate sensitivity changed from S to I, while gatifloxacin changed from I to R in biofield treated LS 10.

Imipenem and meropenem sensitivity changed from susceptible to intermediate and susceptible to resistant, respectively in biofield treated LS 11 as compared to control. Rest of antimicrobials did not show any change in sensitivity pattern after biofield treatment. Antimicrobial resistance can be a result of horizontal gene transfer, and might also have unlinked point mutations of pathogenic genome [26]. Biofield treatment might alter the gene transfer that could lead to alter the sensitivity pattern of tested antimicrobials.

Estimation of minimum inhibitory concentration (MIC)

Biofield treatment on clinical isolates of MDR E. coli showed variation in MIC values with respect to control. MIC values of control and treated group of all the four isolates are presented in Table 2. Biofield treatment has decreased the MIC values of some of the tested antimicrobials such as chloramphenicol (less than 8 µg/mL) that showed two fold decreased MIC value in LS 8, while piperacillin/tazobactam showed four fold decreased MIC value in biofield treated LS 9 as compared to control. Ticarcillin/k-clavulanate also showed decreased MIC value i.e. 64 µg/mL as compared to control in LS 9.

Amikacin, amoxicillin/k-clavulanate, cefotetan, cefoxitin, nitrofurantoin, piperacillin/tazobactam, and ticarcillin/k-clavulanate showed increased MIC values in biofield treated LS 8 and LS 10 with respect to control. Increase in MIC values were also reported in ampicillin/sulbactam and meropenem in LS 8, while gatifloxacin and trimethoprim/sulfamethoxazole in LS 10 as compared to control. Imipenem and meropenem also showed increased MIC value as compared to control in LS 11. An overall 40.63% antimicrobial showed the altered MIC values out of total thirty two tested antimicrobials among four clinical isolates.

All the four isolates had shown different variations in MIC with respect to respective control (Figure 1). Remaining antimicrobials did not show any change in MIC values as compared to their respective control. Best drug prescribed by clinicians against E. coli infections.

Growth of E. coli can be inhibited by a wide range of antimicrobial agents. Clinicians primarily suggest a wide range of antimicrobials, such as ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole against travelers’ diarrhea or associated enteric infections [27]. Antimicrobial resistance is ever-increasing problem at global level, but still fluoroquinolones antimicrobial are the best choice to inhibit the growth of E. coli to treat community and hospital acquired infections [28]. Apart from, fluoroquinolones, carbapenem are effectively used against infections associated with extended-spectrum-β-lactamase (ESBL) producing E. coli. Choice of treatment depends upon the previous history like repeated UTIs, underlying renal pathology, older males, etc. Retamar et al. studied the impact of piperacillin/tazobactam and its MIC value in bacteremia patients, due to ESBL producing E. coli [29]. They conclude that carbapenem are still the best drug of choice to treat infections of ESBL producing Enterobacteriaceae [29]. Biofield treatment on clinical MDR isolates of E. coli had significantly reduced the MIC value of chloramphenicol (two fold, LS 8), piperacillin/tazobactam (four fold, LS 9), and ticarcillin/k-clavulanate (LS 9), as compared to its respective control. Biofield treatment might act on enzymatic or genetic level which might affect the beta-lactamasms production that may lead to alter the sensitivity pattern of tested antimicrobials.

Biochemical and biotype number study

Biochemical study was performed to test the change in biochemical reactions among thirty three biochemicals after biofield treatment. Results of control and treated isolates are summarized in Table 3. Overall biochemical reactions showed 39.39% change in thirty three biochemical reactions, as alteration in percentage value among four isolates with respect to biochemical reactions vary with respect to its control (Figure 1). Adonitol, citrate, esculin hydrolysis, nitrofurantoin, inositol, malonate, tartrate, and urea showed (-) negative to (+) positive reaction, while indole showed (+) positive to (-) negative reaction in LS 8 as compared to control. Hydrogen sulfide showed positive reaction and indole showed negative reaction in biofield treated LS 9. Cetrimide and nitrofurantoin showed positive reaction after biofield treatment in LS 10, while ornithine and raffinose showed positive reaction in LS 11 as compared to control. Indole, nitrate, glucose, and lactose are the positive reaction tests, while Voges-Proskauer, and urea are the typical negative biochemical reaction test of E. coli. Our experimental biochemical reactions in control isolates are well supported with literature [30,31]. Rest of biochemicals did not show any alteration in their reaction after biofield treatment.
| S No. | Code | Biochemical | LS 8 | LS 9 | LS 10 | LS 11 |
|-------|------|-------------|------|------|-------|-------|
|       |      |             | C    | T    | C     | T     |
| 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   | Penicilllin | +    | +    | +     | +     |
| 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 | - | - | - | - | - |

Table 3: Effect of biofield treatment on multidrug resistant lab isolates of *Escherichia coli* to the vital processes occurring in living organisms. C: Control; T: Treatment; LS: Lab Isolate; -: Negative; +: Positive.
Based on the altered biochemical reactions in control and treated groups, biotype numbers were observed using MicroScan Walk-Away® using NBPC 30 panel system, which will detect the change in biochemical reactions, and report the maximum probability of organism on the basis of its biotype number. Out of four tested lab isolates, three had shown change in biotype number after biofield treatment. LS 8 showed changed in biotype number, 7774 5272 as compared to its control, (7711 5012), while in LS 9, altered biotype number was 7352 5072 after biofield treatment as compared to control, 7351 5072. LS 11 showed altered biotype number 5711 5012, as compared to control biotype (5311 4012). Maximum probability of new organism was identified as *Enterobacter aerogenes* in LS 8 after biofield treatment on day 10 with respect to control organism, *E. coli* (Table 4). LS 10 isolate did not show any alteration in biotype number after treatment. Biofield treatment on pathogenic microorganisms had been reported to alter the biochemical reactions, followed by change in biotype number and identification of new microorganism. Current results are well corroborated with reported studies [21,22].

### Table 4: Effect of biofield treatment on biotype number of multidrug resistant lab isolates of *Escherichia coli*.

| Isolate | Group | Biotype Number | Organism Identification |
|---------|-------|----------------|-------------------------|
| LS 8    | C     | 7711 5012      | *E. coli*               |
|         | T     | 7774 5272      | *Enterobacter aerogenes* |
| LS 9    | C     | 7351 5072      | *E. coli*               |
|         | T     | 7352 5072      | *E. coli*               |
| LS 10   | C     | 7311 5012      | *E. coli*               |
|         | T     | 7311 5012      | *E. coli*               |
| LS 11   | C     | 5311 4012      | *E. coli*               |
|         | T     | 5711 5012      | *E. coli*               |

Biofield treatment is included in energy medicine under CAM with increasing number of patients getting benefitted after this therapy [11,32]. Mr. Trivedi's biofield treatment in pathogenic microbes were extensively studied and had shown a significant alteration in the antimicrobial sensitivity pattern, biochemical reactions, and biotype number [21,22]. Results of study conclude that, biofield treatment might be an alternative approach to alter the antimicrobial sensitivity. Mechanism of action through which biofield act on pathogenic microbes, is unknown and needed to explore through in- depth research work. It can be hypothesized from the outcomes of the study that biofield might act on receptor protein interaction of the bacterial cell wall, which may result in altering the sensitivity of antimicrobial after treatment [33].

### Conclusion

The overall observations showed that, Mr Trivedi's biofield treatment on MDR isolates of *E. coli* induced significant alteration in antimicrobial susceptibility pattern, MIC values, biochemical reactions, and biotype number. A fourfold and twofold decrease in MIC values were found in piperacillin/tazobactam, and chloramphenicol after biofield treatment in LS 9 and LS 8 respectively. A significant change in biochemical reactions and biotype numbers were also observed after biofield treatment in clinical isolates of *E. coli*. Based on the study outcome, Mr Trivedi's biofield treatment could be applied to alter the sensitivity pattern of antimicrobials, against multidrug resistance isolates of *E. coli*.

### Acknowledgement

Authors gratefully acknowledged the whole team of PD Hinduja National Hospital and MRC, Mumbai, Microbiology Lab for their support. Authors gratefully acknowledge the support of Trivedi Science, Trivedi Master Wellness and Trivedi Testimonials in this research work.

### References

1. Belanger L, Garennaux A, Harel J, Boulianne M, Nadeau E, et al. (2011) *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. FEMS Immunol Med Microbiol 62: 1-10.
2. Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic *Escherichia coli*. Nat Rev Microbiol 2: 123-140.
3. Russo TA, Johnson JR (2003) Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes Infect 5: 449-456.
4. Furyk JS, Swann O, Molyneux E (2011) Systematic review: neonatal meningitis in the developing world. Trop Med Int Health 16: 672-679.
5. Bonacorsi S, Bingen E (2005) Molecular epidemiology of *Escherichia coli* causing neonatal meningitis. Int J Med Microbiol 295: 373-381.
6. Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA (2001) Multidrug-resistant urinary tract isolates of *Escherichia coli*: prevalence and patient demographics in the United States in 2000. Antimicrob Agents Chemother 45: 1402-1406.
7. Coates A, Hu Y, Bax R, Page C (2002) The future challenges facing the development of new antimicrobial drugs. Nat Rev Drug Discov 1: 895-910.
8. Bartoloni A, Pallecchi I, Benedetti M, Fernandez C, Vallejos Y, et al. (2006) Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. Emerg Infect Dis 12: 907-913.
9. Lucchetti G, de Oliveira RF, Gonçalves JP, Ueda SM, Mimica LM, et al. (2013) Effect of Spiritist ‘passe’ (Spiritual healing) on growth of bacterial cultures. Complement Ther Med 21: 627-632.
10. 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.
11. Barnes PM, Powell-Griner E, McFann K, Nahin RL (2004) Complementary and alternative medicine use among adults: United States, 2002. Adv Data 1-19.
12. Cohl H, Kostyk N, Isokpehi R, Rajnarayanan R (2009) Bio-electrographic method for preventive health care, in proceedings of the 1st IEEE Annual Bioscience and Biotechnology Conference.
13. Movafaghi Z, Farsi M (2009) Biofield therapies: biophysical basis and biological regulations? Complement Ther Clin Pract 15: 35-37.
14. Trivedi MK, Tallapragada RR (2008) A transcendental to changing metal powders. Bull Mater Sci 32: 471-479.
15. Dabbade 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.
16. Trivedi MK, Nayak G, Patil S, Tallapragada RM, Latiyo O (2015) Studies of the atomic and crystalline characteristics of ceramic oxide nano powders after biofield treatment. Ind Eng Manage 4: 161.
17. Shinde V, Sances F, Patil S, Spence A (2012) Impact of biofield treatment on growth and yield of lettuce and tomato. Aust J Basic Appl Sci 6: 100-105.
18. 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.
19. Lenessen AW (2013) Biofield and fungicide seed treatment influences on soybean productivity, seed quality and weed community. Agricultural Journal 8: 138-143.
20. Nayak G, Altekar N (2015) Effect of biofield treatment on plant growth and adaptation. J Environ Health Sci 1: 1-9.
21. Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2013) Phenotypic and biotypic characterization of Klebsiella oxytoca: An impact of biofield treatment. J MicroBiochem Technol 7: 203-206.
22. Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) An effect of biofield treatment on Multidrug-resistant Burkholderia cepacia: A multitroph pathogen. J Trop Dis 3: 167.
23. Fader RC, Weaver E, Fossett R, Toyras M, Vanderlaan J, et al. (2013) Ultilaboratory 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. Themmozi S, Moorthy M, Sureshkumar BT, Suresh M (2014) Antibiotic resistance mechanism of ESBL producing Enterobacteriaceae in clinical field: A review. Int J Pure Appl Biosci 2: 207-226.
25. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18: 657-686.
26. Lee J, Oh CE, Choi EH, Lee HJ (2013) The impact of the increased use of piperacillin/tazobactam on the selection of antibiotic resistance among invasive Escherichia coli and Klebsiella pneumoniae isolates. Int J Infect Dis 17: e638-e643.
27. Hill DR, Beeching NJ (2010) Travelers' diarrhea. Curr Opin Infect Dis 23: 481-487.
28. Pitout JD (2012) Extraintestinal pathogenic Escherichia coli: an update on antimicrobial resistance, laboratory diagnosis and treatment. Expert Rev Anti Infect Ther 10: 1165-1176.
29. Retamar P, López-Cerero L, Muníain MA, Pascual Á, Rodríguez-Baño J; ESBL-REIPI/GEIH Group (2013) Impact of the MIC of piperacillin-tazobactam on the outcome of patients with bacteremia due to extended-spectrum-ß-lactamase-producing Escherichia coli. Antimicrob Agents Chemother 57: 3402-3404.
30. Farasat T, Bilal Z, Yunus F (2012) Isolation and biochemical identification of Escherichia coli from wastewater effluents of food and beverage industry. J Cell Mol Biol 10: 13-18.
31. Singh P, Prakash A (2008) Isolation of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes from milk products sold under market conditions at Agra region. Acta Agriculturae Slovenica 92: 83-88.
32. Clarke TC, Black LI, Stussman BJ, Barnes PM, Nahin RL (2015) Trends in the use of complementary health approaches among adults: United States, 2002-2012. Natl Health Stat Report : 1-16.
33. 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.