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

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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*. Ticarcillin/k-clavulanate showed improved sensitivity (R → I) with decreased MIC value in LS 9 as compared to control. A fourfold and twofold decreased 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 aerogenes* 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 pylonephritis, 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, ceftazidime, 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, ceftazidime 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].

S. No	Antimicrobial	LS 8		LS 9		LS 10		LS 11	
		C	T	C	T	C	T	C	T
1	Amikacin	S	R	R	R	S	R	S	S
2	Amoxicillin/k-clavulanate	S	I	I	I	S	R	R	R
3	Ampicillin/sulbactam	I	R	R	R	R	R	R	R
4	Ampicillin	R	R	R	R	R	R	R	R
5	Aztreonam	EBL?	R	EBL?	EBL?	EBL?	EBL?	EBL?	EBL?
6	Cefazolin	R	R	R	R	R	R	R	R
7	Cefepime	R	R	R	R	R	R	R	R
8	Cefotaxime	EBL?	R	EBL?	EBL?	EBL?	EBL?	EBL?	EBL?
9	Cefotetan	S	I	S	S	S	R	R	R
10	Cefoxitin	I	R	R	R	S	R	R	R
11	Ceftazidime	EBL?	R	EBL?	EBL?	EBL	EBL?	EBL?	EBL?
12	Ceftriaxone	EBL?	R	EBL?	EBL?	EBL	EBL?	EBL?	EBL?
13	Cefuroxime	R	R	R	R	R	R	R	R
14	Cephalothin	R	R	R	R	R	R	R	R
15	Chloramphenicol	I	S	R	R	R	R	R	R
16	Ciprofloxacin	R	R	R	R	R	R	R	R
17	ESBL-a Scrn	EBL?	-	EBL?	EBL?	EBL?	EBL?	EBL?	EBL?
18	ESBL-b Scrn	EBL?	-	EBL?	EBL?	EBL?	EBL?	EBL?	EBL?
19	Gatifloxacin	R	R	R	R	I	R	R	R
20	Gentamicin	R	R	R	R	R	R	R	R
21	Imipenem	S	S	S	S	S	S	S	I
22	Levofloxacin	R	R	R	R	R	R	R	R
23	Meropenem	S	I	S	S	S	S	S	R
24	Moxifloxacin	R	R	R	R	R	R	R	R
25	Nitrofurantoin	-	-	-	-	-	-	-	-
26	Norfloxacin	-	-	-	-	-	-	-	-
27	Piperacillin/tazobactam	S	R	I	S	S	I	R	R
28	Piperacillin	R	R	R	R	R	R	R	R
29	Tetracycline	R	R	R	R	R	R	R	R
30	Ticarcillin/k-clavulanate	S	R	R	I	S	I	R	R
31	Tobramycin	R	R	R	R	R	R	R	R
32	Trimethoprim/sulfamethoxazole	R	R	R	R	S	R	R	R

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	
		C	T	C	T	C	T	C	T
1	Amikacin	≤16	>32	>32	>32	≤16	>32	≤16	≤16
2	Amoxicillin/k-clavulanate	≤8/4	16-Aug	16-Aug	16-Aug	≤8/4	>16/8	>16/8	>16/8
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	≤16	≤16	>32	>32	>32
10	Cefoxitin	16	>16	>16	>16	≤8	>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	Gatifloxacin	>4	>4	>4	>4	4	>4	>4	>4
20	Gentamicin	>8	>8	>8	>8	>8	>8	>8	>8
21	Imipenem	≤4	≤4	≤4	≤4	≤4	≤4	≤4	8
22	Levofloxacin	>4	>4	>4	>4	>4	>4	>4	>4
23	Meropenem	≤4	8	≤4	≤4	≤4	≤4	≤4	>8
24	Moxifloxacin	>4	>4	>4	>4	>4	>4	>4	>4
25	Nitrofurantoin	≤32	>64	≤32	≤32	≤32	>64	≤32	≤32
26	Norfloxacin	>8	>8	>8	>8	>8	>8	>8	>8
27	Piperacillin/tazobactam	≤16	>64	64	≤16	≤16	64	>64	>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	>64	64	≤16	64	>64	>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

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

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 leads to resistant in case of aztreonam, cefotaxime, 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 LS 10 after 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.

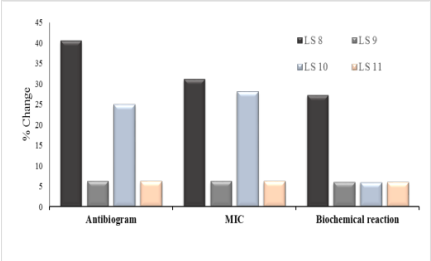

Figure 1: Percentage change in antimicrobial sensitivity pattern, minimum inhibitory concentrations and biochemical reactions after biofield treatment in multi-drug resistant lab isolates of *Escherichia coli*.

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, carbapenems 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 carbapenems 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-lactamases 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	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	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	-	-	-	-	-	-	-	-

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 biotype number and identification of new microorganism. Current results are well corroborated with reported studies [21,22].

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*

Table 4: Effect of biofield treatment on biotype number of multidrug resistant lab isolates of *Escherichia 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 results 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 multi-drug resistance isolates of *E. coli*.

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