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Antibiogram Typing and Biochemical Characterization of *Klebsiella pneumoniae* after Biofield Treatment

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Abstract

Klebsiella pneumoniae (*K. pneumoniae*) is a common nosocomial pathogen causing respiratory tract (pneumoniae) and blood stream infections. Multidrug-resistant (MDR) isolates of *K. pneumoniae* infections are difficult to treat in patients in health care settings. Aim of the present study was to determine the impact of Mr. Trivedi's biofield treatment on four MDR clinical lab isolates (LS) of *K. pneumoniae* (LS 2, LS 6, LS 7, and LS 14). Samples were divided into two groups *i.e.* control and biofield treated. Control and treated groups were analyzed for antimicrobial susceptibility pattern, minimum inhibitory concentration (MIC), biochemical study and biotype number using MicroScan Walk-Away® system. The analysis was done on day 10 after biofield treatment as compared with control group. Antimicrobial sensitivity assay showed that there was 46.42% alteration in sensitivity of tested antimicrobials in treated group of MDR *K. pneumonia* isolates. MIC results showed an alteration in 30% of tested antimicrobials out of thirty after biofield treatment in clinical isolates of *K. pneumoniae*. An increase in antimicrobial sensitivity and decrease in MIC value was reported (in LS 6) in case of piperacillin/tazobactam and piperacillin. Biochemical study showed a 15.15% change in biochemical reactions as compared to control. A significant change in biotype numbers were reported in all four clinical isolates of MDR *K. pneumoniae* after biofield treatment as compared to control group. On the basis of changed biotype number after biofield treatment, new organism was identified as *Enterobacter aerogenes* in LS 2 and LS 14. These results suggest that biofield treatment has a significant effect on altering the antimicrobial sensitivity, MIC values, biochemical reactions and biotype number of multidrug-resistant isolates of *K. pneumoniae*.

Keywords: *Klebsiella pneumoniae*; Biofield Treatment; Multidrug-Resistant; Antimicrobial susceptibility; Biochemical Reaction; Biotyping

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is Gram-negative, rod-shaped, facultative anaerobic, and nonmotile bacterium, belongs to family *Enterobacteriaceae*. It is a common human pathogen associated with nosocomial and community infections [1]. *K. pneumoniae* isolates causes several infections such as pneumonia, septicemia, wound infections, and urinary tract infections, which ultimately lead to morbidity and mortality especially in immunocompromised patients, and patients of intensive care units, pediatrics and surgical wards [2]. *K. pneumoniae* acquire resistance against existing antimicrobials by multiple mechanism results in increased multidrug-resistant (MDR) of *K. pneumoniae* that leads to serious problem in hospital settings and health concern. Emergence of resistance occurs not only in MDR isolates but also exist in pan-drug resistant (PDR) isolates of *K. pneumoniae*. PDR refers to the resistant strains those are specifically resistant to 7 antimicrobial agents such as cefepime, imipenem, meropenem, ceftazidime, ciprofloxacin, piperacillin-tazobactam, and levofloxacin [3]. Apart from this, the extended-spectrum β-lactamase (ESBL) producing *Klebsiella* from a patient has been identified which causes serious threat worldwide [4,5]. Continuous use of antibiotics leads to resistance in microorganisms *via.* different pathways mediated by plasmids, transposons, and gene cassettes in integrons [6,7]. Carbapenem is usually preferred for the infection caused by MDR isolates of *K. pneumoniae* but recently carbapenem-resistant *K. pneumoniae* was also reported [8]. Due to dramatically increase in drug resistant in *K. pneumoniae*, very few treatment options are available. Alternative approaches are available but altering the sensitivity pattern of antimicrobials using biofield is not available against MDR microorganism, apart from existing allopathic system of medicine. Biofield treatment may be an alternative approach to alter the susceptibility pattern of *K. pneumoniae.* Complementary and alternative medicine (CAM) therapies are commonly practiced in healthcare sector and about 36% of Americans regularly uses some form of CAM [9]. CAM include numerous energy therapies, biofield therapy, is one of the energy medicine widely used worldwide to improve the human health. The energy exists in various forms that can be produced from different sources such as potential, electrical, kinetic, magnetic, and nuclear energy. However, electromagnetic field defines as when electrical signals fluctuate will generate magnetic field with respect to time. The cumulative effect of bio-magnetic and electric field that surrounds the human body is defined as biofield. The biofield energy can be monitored by using electromyography (EMG), electrocardiography (ECG) and electroencephalogram (EEG) [10]. According to Lucchetti *et al.* biofield energy has shown significant effect on growth of bacterial cultures [11]. Mr. Trivedi has the ability to harness the energy from environment or universe and can transmit into any living or nonliving 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®. Mr. Trivedi's biofield treatment was extensively studied in different fields such as in material science [12,13], agricultural science [14-16], and in biotechnology [17]. Further,

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the biofield treatment has considerably altered the susceptibility of antimicrobials and biotype of microbes [18-20]. By considering the above mentioned facts and literature reports on biofield treatment, the present work was undertaken to evaluate the impact of biofield treatment on antimicrobials susceptibility, biochemical reactions pattern, and biotype of MDR isolates of *K. pneumoniae*.

Materials and Methods

Experimental design and bacterial isolates

MDR clinical lab isolates (*i.e.* LS 2, LS 6, LS 7 and LS 14) of *K. pneumoniae* were obtained from stored stock cultures in Microbiology Lab, Hinduja Hospital, Mumbai. Each MDR strains was divided into two groups *i.e.* control and treatment. The acceptability of the identification media and antimicrobial agents were checked prior to the study on microorganisms. The antimicrobial susceptibility, biochemical reactions, and biotype number were evaluated on MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA) using Negative Breakpoint Combo 30 (NBPC 30) panel. The NBPC 30 panel was stored at 2 to -25°C. All antimicrobials and biochemicals were procured from Sigma Aldrich, USA.

Biofield treatment strategy

Treatment groups of each strain, 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 analyzed on day 10 using the standard protocols. The study was conducted on automated MicroScan Walk-Away® system (Dade Behring Inc., USA).

Evaluation of antimicrobial susceptibility assay

Antimicrobial susceptibility patterns of MDR lab isolates of *K. pneumoniae* were studied using MicroScan Walk-Away® using Negative Break Point Combo (NBPC 30) panel as per manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate, IB: Inducible β-lactamase; EBL: Suspected extended-spectrum β -lactamases, and R: Resistant) and MIC values were determined by observing the lowest antimicrobial concentration showing growth inhibition [21]. The antimicrobials used in the susceptibility assay *viz.* amikacin, amoxicillin/k-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, cefotetan, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, norfloxacin, nitrofurantoin piperacillin, piperacillin/tazobactam, tetracycline, ticarcillin/K-clavulanate, tobramycin, and trimethoprim/sulfamethoxazole

Biochemical study

Biochemical studies of each MDR isolates of *K. pneumoniae* were determined by MicroScan Walk-Away® using NBPC 30 panel system in both control and treated groups. Biochemicals used in the study are acetamide, adonitol, arabinose, arginine, cetrimide, cephalothin, citrate, colistin, esculin hydrolysis, nitrofurantoin, glucose, hydrogen sulfide, indole, inositol, kanamycin, lysine, malonate, melibiose, nitrate, oxidation-fermentation, galactosidase, ornithine, oxidase, penicillin, raffinose, rhamnose, sorbitol, sucrose, tartarate, tryptophan deaminase, tobramycin, urea, and Voges-Proskauer [21].

Identification by biotype number

The biotype number of each MDR isolates of *K. pneumoniae* 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 [21].

Results and Discussion

Antimicrobial susceptibility study

Results of antimicrobial sensitivity pattern and MIC values of control and treated MDR isolates of *K. pneumoniae* are summarized in Tables 1 and 2, respectively. All these changes were observed on 10 days after biofield treatment as compared to control group. Overall, 46.42% of tested antimicrobials out of twenty eight, showed alteration in antimicrobial sensitivity pattern against biofield treated MDR isolates of *K. pneumoniae*. All four MDR isolates, showed variations in antimicrobial sensitivity assay *viz.* 32.14% in LS 2, 25% in LS 6, 17.85% in LS 7, and 28.57% in LS 14 against the tested antimicrobials (Figure 1). Extended spectrum beta-lactamases (ESBLs) are rapidly evolved group of beta-lactamases enzyme, which confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, monobactam and aztreonam. Apart from beta-lactam antibiotics, ESBLs are also resistant to other classes of non-penicillin antibiotics [22]. Beta-lactamases are enzymes that inactivates the antibiotic and are present in almost all Gram-negative bacilli. However, some pathogenic species, such as *E. coli* and *Klebsiella* spp., are not able to induce the production of β-lactamase which varies from low to high level. In some species, exposure to β-lactams will induced the production level of β-lactamase, commonly results in resistance to these agents. These inducible β-lactamases are frequently found in Enterobacter spp. [23]. Experimental results of antimicrobial sensitivity assay showed altered sensitivity pattern in biofield treated clinical isolates of *K. pneumonia*. Aztreonam, cefotaxime, ceftazidime, and ceftriaxone sensitivity changed from EBL → R in LS 2 and LS 14. Sensitivity of amoxicillin/k-clavulanate changed from S → R in LS 7 and LS 14, while S → IB and I → R in LS 2 and LS 6 respectively. Cefotetan and cefoxitin found changed sensitivity pattern from S → R in LS 7, and while S → IB in LS 2. Sensitivity of cefotetan changed from I → R in LS 6 while S → IB in LS 14. Chloramphenicol and imipenem sensitivity changed from S → R in LS 6. Although, in imipenem sensitivity changed from S → I in LS 6. Meropenem sensitivity changed from I → R and S → R in LS 6 and LS 7 respectively. An increase in sensitivity was reported in piperacillin/tazobactam and piperacillin *i.e.* from R → I in biofield treated LS 6 as compared to control. Although, piperacillin/tazobactam sensitivity changed from S → IB and S → I in LS 2 and LS 14 respectively. Ticarcillin/K-clavulanate sensitivity altered from S → I and S → R in LS 2 and LS 14 respectively (Table 1). Rest of antimicrobials did not show any change in sensitivity pattern after biofield treatment.

Determination of Minimum Inhibitory Concentration (MIC)

MIC values of all the clinical MDR isolates of control and biofield treated *K. pneumoniae* are summarized in Table 2. MIC values were decreased in case of piperacillin/tazobactam and piperacillin in LS 6 isolate only, while in rest of the antimicrobials, MIC values were increased as compared to control. Overall, MIC results showed an alteration in 30% tested antimicrobials (*i.e.* nine out of thirty) after biofield treatment in clinical isolates of *K. pneumoniae* (Table 2 and Figure 1). A decreased in MIC values in piperacillin/tazobactam and piperacillin were reported along with increases antimicrobial sensitivity after biofield treatment (64 µg/ml) in LS 6. Current treatment strategy

S. No.	Antimicrobial	LS 2		LS 6		LS 7		LS 14	
		C	T	C	T	C	T	C	T
1	Amikacin	S	S	R	R	R	R	S	S
2	Amoxicillin/k-clavulanate	S	IB	I	R	S	R	S	R
3	Ampicillin/sulbactam	R	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	R
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	R
9	Cefotetan	S	IB	I	R	S	R	S	IB
10	Cefoxitin	S	IB	R	R	S	R	R	R
11	Ceftazidime	EBL	R	EBL	EBL	EBL	EBL	EBL	R
12	Ceftriaxone	EBL	R	EBL	EBL	EBL	EBL	EBL	R
13	Cefuroxime	R	R	R	R	R	R	R	R
14	Cephalothin	R	R	R	R	R	R	R	R
15	Chloramphenicol	S	S	S	R	R	R	I	I
16	Ciprofloxacin	S	S	R	R	R	R	R	R
17	Gatifloxacin	S	S	R	R	R	R	R	R
18	Gentamicin	R	R	R	R	R	R	R	R
19	Imipenem	S	S	S	R	S	I	S	S
20	Levofloxacin	S	S	R	R	R	R	R	R
21	Meropenem	S	S	I	R	S	R	S	S
22	Moxifloxacin	S	S	R	R	R	R	R	R
23	Piperacillin	R	R	R	I	R	R	R	R
24	Piperacillin/tazobactam	S	IB	R	I	R	R	S	I
25	Tetracycline	S	S	R	R	R	R	R	R
26	Ticarcillin/k-clavulanate	S	I	R	R	R	R	S	R
27	Tobramycin	R	R	R	R	R	R	R	R
28	Trimethoprim/sulfamethoxazole	S	S	R	R	R	R	R	R

C: Control; T: Treatment; R: Resistant; I: Intermediate; S: Susceptible; LS: Lab Isolate; EBL: Suspected extended-spectrum beta-lactamases

Table 1: Effect of biofield treatment on multidrug resistant lab isolates of *Klebsiella pneumoniae* to antimicrobial susceptibility.

against *K. pneumoniae* infections preferably uses cefoperazone/sulbactam, piperacillin/tazobactam, and imipenem antimicrobials [24]. Although, piperacillin/tazobactam antimicrobial agent is useful and preferred in neonatal infections caused due to *K. pneumoniae* [25]. Biofield treatment in clinical isolate (LS 6) significantly increased the sensitivity and decreased the MIC values of piperacillin/tazobactam and piperacillin. In *Enterobacteriaceae* family, the most prevalent mechanism of acquired resistance in β-lactam antibiotics (piperacillin/tazobactam and piperacillin) are the production of β-lactamases [26]. Biofield treatment might act on enzymatic or genetic level which might affects the β-lactamases production that may lead to alter the sensitivity pattern of tested antimicrobials.

Biochemical and biotype number study

Biochemical study results of control and biofield treated groups are summarized in Table 3 and Figure 1. Results showed that overall 15.15% change in tested biochemical reactions among 4 treated MDR clinical isolates of *K. pneumoniae* as compared to control. Cetrimide changed from (-) negative to (+) positive reaction in LS 6, LS 7, and LS 14 as compared to control. Voges-Proskauer changed from (+) positive to (-) negative reaction in LS 2, LS 6, and LS 7. Indole changed from (+) positive to (-) negative reaction in LS 7. Ornithine changed from negative (-) to positive (+) reaction in LS 14. Rest of biochemicals did not show any alteration in their reaction after biofield treatment. Voges-Proskauer, citrate, arabinose, lysine, glucose, sucrose, malonate are the standard positive reaction biochemical tests of *K. pneumoniae* while hydrogen sulfide, indole, ornithine and cetrimide are the standard negative reaction test. Biochemical reactions of control MDR isolates of *K. pneumoniae* were well supported with literature data [27]. Based on the biochemical results, significant alteration in biotype numbers were observed in all the four biofield treated lab isolates *i.e.* LS 2, LS 6, LS 7, and LS 14 as compared to control. New organism was identified as *Enterobacter aerogenes* in LS 2 and LS 14 after biofield treatment on day 10 with respect to control (Table 4). Biofield treatment as an alternate and complementary medicine, increasingly used in biomedical health care system such as reduction in pain and anxiety [28]. However, National Center for Complementary and Alternative Medicine/National Institute of Health (NCCAM/NIH), now defined biofield therapies in subcategory of energy therapies as one of the five complementary medicine domain [29]. Mr. Trivedi's biofield treatment in pathogenic microbes were extensively studied and had shown significant alteration in the antimicrobial sensitivity pattern, biochemical reactions, and biotype number [18,19]. Biofield treatment might be responsible to do 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 also alter that could lead to show different phenotypic characteristics. Hence a cascade of intra-cellular signals may be initiated, accelerated or inhibited [30]. The overall observations showed that, biofield treatment on MDR isolates of *K. pneumoniae* induced significant alteration in antimicrobial susceptibility pattern, MIC values, biochemical reactions, and biotype number.

S. No.	Antimicrobial	LS 2		LS 6		LS 7		LS 14	
		C	T	C	T	C	T	C	T
1	Amikacin	≤16	≤16	>32	>32	>32	>32	≤16	≤16
2	Amoxicillin/ Clavulanic acid	≤8/ 4	≤8/4	16/8	>16/8	≤8/4	>16/8	≤8/4	>16/8
3	Ampicillin/Sulbactam	>16/8	>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	≤16	32	>32	≤16	>32	≤16	≤16
10	Cefoxitin	≤8	≤8	>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	≤8	≤8	≤8	>16	>16	>16	16	16
16	Ciprofloxacin	≤1	≤1	>2	>2	>2	>2	>2	>2
17	Gatifloxacin	≤2	≤2	>4	>4	>4	>4	>4	>4
18	Gentamicin	>8	>8	>8	>8	>8	>8	>8	>8
19	Imipenem	≤4	≤4	≤4	>8	≤4	8	≤4	≤4
20	Levofloxacin	≤2	≤2	>4	>4	>4	>4	>4	>4
21	Meropenem	≤4	≤4	8	>8	≤4	>8	≤4	≤4
22	Moxifloxacin	≤2	≤2	>4	>4	>4	>4	>4	>4
23	Nitrofurantoin	≤32	≤32	>64	>64	>64	>64	≤32	≤32
24	Norfloxacin	≤4	≤4	>8	>8	>8	>8	>8	>8
25	Piperacillin	>64	>64	>64	64	>64	>64	>64	>64
26	Piperacillin/Tazobactam	≤16	≤16	>64	64	>64	>64	≤16	64
27	Tetracycline	≤4	≤4	>8	>8	>8	>8	>8	>8
28	Ticarcillin/K-Clavulanate	≤16	64	>64	>64	>64	>64	≤16	>64
29	Tobramycin	>8	>8	>8	>8	>8	>8	>8	>8
30	Trimethoprim/Sulfamethoxazole	≤2/38	≤2/38	>2/38	>2/38	>2/38	>2/38	>2/38	>2/38

MIC values are presented in µg/ml; C: Control; T: Treatment; LS: Lab Isolate

Table 2: Minimum inhibitory concentration (MIC) of multidrug resistant lab isolates of *Klebsiella pneumoniae*.

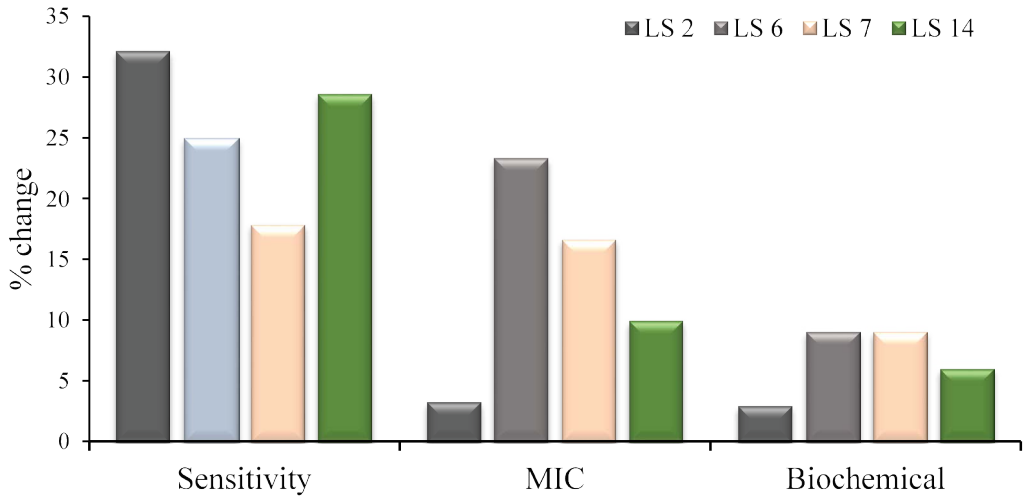

Figure 1: Percentage change in antimicrobial sensitivity pattern, minimum inhibitory concentrations and biochemical reactions after biofield treatment in multi-drug resistant lab isolates of *Klebsiella pneumonia.*

S. No.	Code	Biochemical	LS 2		LS 6		LS 7		LS 14	
			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	+	-	+	-	+	-	+	+

C: Control; T: Treatment; LS: Lab Isolate; -: Negative; +: Positive

Table 3: Effect of biofield treatment on multidrug resistant lab isolates of *Klebsiella pneumoniae* to the vital processes occurring in living organisms.

Isolate	Group	Biotype Number	Organism Identification
LS 2	C	7770 4372	*K. pneumoniae*
	T	7770 4272	*Enterobacter aerogenes*
LS 6	C	7774 4372	*K. pneumoniae*
	T	7776 4272	*K. pneumoniae*
LS 7	C	7774 4362	*K. pneumoniae*
	T	7774 4262	*K. pneumoniae*
LS 14	C	7774 4372	*K. pneumoniae*
	T	7774 5372	*Enterobacter aerogenes*

C: Control; T: Treatment; LS: Lab Isolate

Table 4: Effect of biofield treatment on multidrug resistant lab isolates of *Klebsiella pneumoniae* to distinguishing feature of the genotype

Conclusion

Overall data conclude that there has a significant impact of biofield treatment on antimicrobial susceptibility pattern, MIC values, biochemical reactions, and biotype number in all the four clinical MDR lab isolates of *K. pneumoniae*. Based on the study outcome, biofield treatment could be applied to alter the sensitivity pattern of antimicrobials, against multi-drug resistance isolates of *K. pneumoniae*.

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Conflict of Interest

The authors declare that they have no competing interest.

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