

Biofield Treatment: An Alternative Approach to Combat Multidrug-Resistant Susceptibility Pattern of *Raoultella ornithinolytica*

Mahendra Kumar Trivedi¹, Shrikant Patil¹, Harish Shettigar¹, Mayank Gangwar² and Snehasis Jana^{2*}

¹Trivedi Global Inc., 10624 S Eastern Avenue Suite A-969, Henderson, NV 89052, USA

²Trivedi Science Research Laboratory Pvt. Ltd, Madhya Pradesh, India

*Corresponding author: Jana S, Trivedi Science Research Laboratory Pvt. Ltd, Fortune City, Hoshangabad Rd., Bhopal, Madhya Pradesh, India, Tel: +91-755-6660006; E-mail: publication@trivedisrl.com

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Abstract

Raoultella ornithinolytica belongs to the family of *Enterobacteriaceae*, a Gram-negative encapsulated aerobic bacillus associated with bacteremia and urinary tract infections. As biofield therapy is increasingly popular in biomedical health care, so present study aimed to evaluate the impact of Mr. Trivedi's biofield treatment on antimicrobial sensitivity, minimum inhibitory concentration (MIC), biochemical study, and biotype number of multidrug resistant strain of *R. ornithinolytica*. Clinical sample of *R. ornithinolytica* was divided into two groups i.e. control and biofield treated which were analyzed for the above parameters using MicroScan Walk-Away® system on day 10 after treatment. Antimicrobial sensitivity assay results showed a significant increase (60.71%) in sensitivity pattern of antimicrobials i.e. changed from resistant to susceptible while 10.71% of tested antimicrobials changed from intermediate to susceptible as compared to control. MIC results showed a significant decrease in MIC values of 71.88% tested antimicrobials as compared to control.

Biochemical reaction study showed 15.15% alteration in different biochemical such as ceftrimide, cephalothin, kanamycin, and ornithine after biofield treatment as compared to control. A significant change in biotype number (7775 4370) was also observed with organism identified as *Klebsiella oxytoca* after biofield treatment as compared to control (7775 5372). Overall results conclude that biofield treatment could be used as complementary and alternative treatment strategy against multidrug resistant strain of *R. ornithinolytica* with respect to improve the sensitivity and reduce the MIC values of antimicrobials. Hence, it is assumed that biofield treatment might be a suitable cost effective treatment strategy in near future, which could have therapeutic value in patients suffering from multidrug resistant pathogens.

Keywords: *Raoultella ornithinolytica*; Biofield treatment; Antimicrobial susceptibility; Biochemical reaction; Biotype; Multidrug resistant

Introduction

Genus *Raoultella* belongs to the family *Enterobacteriaceae*, mainly contains Gram-negative, aerobic, non-motile, capsulated, and facultative anaerobic bacilli [1]. Initially, genus *Raoultella* was classified in *Klebsiella*, further on the basis of 16S rRNA and rpoB genes study includes it in new genus *Raoultella*. It includes *Raoultella ornithinolytica*, *R. electrica*, *R. planticola*, and *R. terrigena*. *R. ornithinolytica* is widely distributed in aquatic flora, fishes and insects, but it was associated with histamine poisoning in fish [2]. Human infections are often less reported earlier, but different cases of bacteremia are reported due to *R. terrigena* [3], *R. planticola* [4] and *R. ornithinolytica* [5,6].

Although most of the infectious cases are often associated with underlying existing infection especially malignancies. However, other pathogenic cases such as urinary tract infection, soft issue infections in adults and neonatal infections are also reported [7]. Thus, this is very clear from the above reports that *Raoultella* genus acts as human pathogen and multidrug resistant (MDR) strain will cause a serious threat to human health causing pneumonia and other infections [8]. Broad spectrum antimicrobials and combinations therapies are the

only treatment strategy to cure the infection of MDR. Antimicrobials are always associated with serious side effects [9]. Currently, no such alternative therapies apart from medicines are available against MDR microorganism infections, biofield treatment may be a new approach to improve the susceptibility pattern of *R. ornithinolytica*.

The National Center for Complementary and Alternative Medicine (NCCAM) places biofield therapy under subcategory of energy therapies as one of the five complementary medicine domain. It is scientifically preferred term for the biologically produced electromagnetic and subtle energy field that provides regulatory and communication functions within the organism. The cumulative effect of bio-magnetic and electric field that surrounds the human body is defined as biofield energy. However, the energy can exist in several forms such as kinetic, potential, electrical, magnetic, and nuclear. But human body has the power to produce measurable electric and magnetic signals [10,11].

Similarly, the human nervous system consists of the energy and chemical information in the form of electrical signals. 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 via biofield energy and the process is known as biofield treatment. Even every cell of human body will produce minute amount of magnetic and electric field, as it always covers with positive

and negative charge in outer and inner cell wall respectively [12]. The biofield energy can be monitored by using electromyography (EMG), electrocardiography (ECG) and electroencephalogram (EEG) [13]. Mr. Trivedi's biofield treatment is well known and significantly studied in different fields such as altering the sensitivity pattern of different human pathogens [14-16]. It has been significantly studied in field of agriculture [17-19], biotechnology [20,21], and in material science [22-24].

Materials and Methods

Experimental design and biofield treatment

MDR strain of *R. ornithinolytica* was obtained from stored stock cultures of clinical sample in Microbiology Lab, Hinduja Hospital, Mumbai. MDR strain was divided in two groups i.e. control and treatment. In case of treatment group, sealed pack of MDR strain of *R. ornithinolytica* was handed over to Mr. Trivedi for biofield treatment under laboratory conditions. Mr. Trivedi provided the treatment through his energy transmission process to the treated group without touching the sample. The biofield treated sample was returned in the similar sealed condition and further analyzed on day 10 using the standard protocols. After biofield treatment, following parameters like antimicrobial susceptibility, MIC values, biochemical reactions, and biotype number were measured using MicroScan Walk-Away® system (Dade Behring Inc., USA) with respect to control. All antimicrobials and biochemicals were procured from Sigma Aldrich.

Evaluation of antimicrobial susceptibility assay

Antimicrobial susceptibility pattern of *R. ornithinolytica* was studied using MicroScan Walk-Away® system along with Negative Break Point Combo (NBPC 30) panel as per manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate, and R: Resistant) and minimum inhibitory concentration (MIC) values were determined by observing the lowest antimicrobial concentration showing growth inhibition. The antimicrobials used in the susceptibility assay viz. amikacin, amoxicillin/k-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, cefotetan, ceftazidime, ceftazidime, ceftriaxone, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, ESBL-a Scrn, ESBL-b Scrn, gatifloxacin, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, nitrofurantoin, norfloxacin, piperacillin, piperacillin/tazobactam, tetracycline, ticarcillin/k-clavulanate, and tobramycin, trimethoprim/sulfamethoxazole [25].

Biochemical study

Biochemical study of *R. ornithinolytica* was determined using MicroScan Walk-Away® 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, rhaminose, sorbitol, sucrose, tartarate, tryptophan deaminase, tobramycin, urea, and Voges-Proskauer [25].

Identification by biotype number

The biotype number of *R. ornithinolytica* in control and treated samples were determined followed by identification of microorganism by MicroScan Walk-Away® processed panel report with the help of biochemical reaction data [25].

Results

Antimicrobial susceptibility

Results of antimicrobial sensitivity pattern and MIC values of control and biofield treated MDR strain of *R. ornithinolytica* are summarized in Tables 1 and 2, respectively. All these changes were observed on day 10 after the biofield treatment as compared to control group. Antimicrobial sensitivity assay showed that 60.71% of tested 28 antimicrobials were reported with significant increase in sensitivity pattern from R → S viz. amoxicillin/k-clavulanate, ampicillin/sulbactam, aztreonam, cefazolin, cefotaxime, ceftriaxone, cefuroxime, cephalothin, ciprofloxacin, gatifloxacin, gentamicin, levofloxacin, moxifloxacin, piperacillin, tetracycline, trimethoprim/sulfamethoxazole, and tobramycin after biofield treatment. Apart from this, 10.71% tested antimicrobials showed altered sensitivity pattern from I → S in ceftazidime, piperacillin/tazobactam, and ticarcillin/k-clavulanate as compared to control. Rest of the antimicrobials (28.7%) did not show any alteration in sensitivity pattern (Table 1).

S. No.	Antimicrobial	Control	Treated
1	Amikacin	S	S
2	Amoxicillin/k-clavulanate	R	S
3	Ampicillin/sulbactam	R	S
4	Ampicillin	R	R
5	Aztreonam	R	S
6	Cefazolin	R	S
7	Cefepime	S	S
8	Cefotaxime	R	S
9	Cefotetan	S	S
10	Cefoxitin	S	S
11	Ceftazidime	I	S
12	Ceftriaxone	R	S
13	Cefuroxime	R	S
14	Cephalothin	R	S
15	Chloramphenicol	S	S
16	Ciprofloxacin	R	S
17	Gatifloxacin	R	S
18	Gentamicin	R	S
19	Imipenem	S	S
20	Levofloxacin	R	S

21	Meropenem	S	S
22	Moxifloxacin	R	S
23	Piperacillin	R	S
24	Piperacillin/tazobactam	I	S
25	Tetracycline	R	S
26	Ticarcillin/k-clavulanate	I	S
27	Tobramycin	R	S
28	Trimethoprim/sulfamethoxazole	R	S
R: Resistant; I: Intermediate; S: Susceptible			

Table 1: Effect of biofield treatment on *Raoultella ornithinolytica* to antimicrobial susceptibility.

Besides antimicrobial sensitivity assay, an overall significant decrease in MIC values were reported in 71.88% of antimicrobials (twenty three out of thirty two) against *R. ornithinolytica* as compared to control. Four folds decreased in MIC value were observed in case of cefotaxime, ceftriaxone, piperacillin, piperacillin/tazobactam, ticarcillin/k-clavulanate, and cefuroxime while two folds decreased in MIC value in case of amoxicillin/clavulanic acid, ampicillin/sulbactam, aztreonam, cefazolin, cephalothin, ceftazidime, gentamicin, norfloxacin, tetracycline, tobramycin, gatifloxacin, levofloxacin, moxifloxacin, and ciprofloxacin. Trimethoprim/sulfamethoxazole was reported with decreased MIC value (less than 2/38 µg/mL) as compared to control (Figure 1 and Table 2).

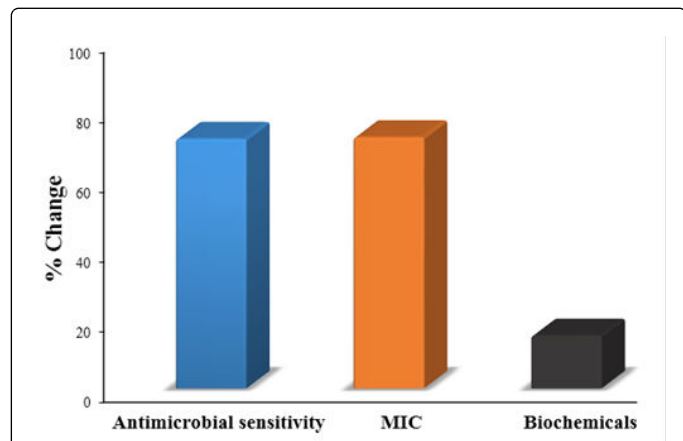


Figure 1: Percentage change in antimicrobial sensitivity pattern, minimum inhibitory concentration (MIC) values and biochemical reactions after biofield treatment of multidrug resistant strain of *Raoultella ornithinolytica*.

S. No.	Antimicrobial	Control (µg/mL)	Treated (µg/mL)
1	Amikacin	≤16	≤16
2	Amoxicillin/k-clavulanate	>16/8	≤8/4
3	Ampicillin/sulbactam	>16/8	≤8/4

4	Ampicillin	>16	>16
5	Aztreonam	>16	≤8
6	Cefazolin	>16	≤8
7	Cefepime	≤8	≤8
8	Cefotaxime	>32	≤8
9	Cefotetan	≤16	≤16
10	Cefoxitin	≤8	≤8
11	Ceftazidime	16	≤8
12	Ceftriaxone	>32	≤8
13	Cefuroxime	>16	≤4
14	Cephalothin	>16	≤8
15	Chloramphenicol	≤8	≤8
16	Ciprofloxacin	>2	≤1
17	ESBL-a Scrn	>4	≤4
18	ESBL-b Scrn	>1	≤1
19	Gatifloxacin	>4	≤2
20	Gentamicin	>8	≤4
21	Imipenem	≤4	≤4
22	Levofloxacin	>4	≤2
23	Meropenem	≤4	≤4
24	Moxifloxacin	>4	≤2
25	Nitrofurantoin	≤32	≤32
26	Norfloxacin	>8	≤4
27	Piperacillin	>64	≤16
28	Piperacillin/tazobactam	64	≤16
29	Tetracycline	>8	≤4
30	Ticarcillin/k-clavulanate	64	≤16
31	Tobramycin	>8	≤4
32	Trimethoprim/sulfamethoxazole	>2/38	≤2/38

ESBL-a, b Scrn: Extended-Spectrum β-Lactamase screen.

Table 2: Minimum inhibitory concentration (MIC) of tested antimicrobials on *Raoultella ornithinolytica*.

Biochemical reaction

Table 3 summarizes the data related to biochemical reactions in control and biofield treated group. Results showed that, 15.15% tested biochemical reactions out of 33 were changed from positive (+) to negative (-) such as cetrime, cephalothin, kanamycin, ornithine, and tobramycin after biofield treatment as compared with control (Figure 1).

S. No.	Code	Biochemical	Control	Treated
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	Rhaminose	+	+
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)

Table 3: Effect of biofield treatment on *Raoultella ornithinolytica* to the vital process of microorganism.

Organism identification by biotype number

Based on the biochemical results, change in biotype number was observed in biofield treated group (7775 4370) at day 10 of *R. ornithinolytica* with respect to control (7775 5372). After alteration in biotype number the new organism was identified as *Klebsiella oxytoca* (Table 4).

Feature	Control	Treated
Biotype	77755372	77754370
Organism Identification	<i>Raoultella ornithinolytica</i>	<i>Klebsiella oxytoca</i>

Table 4: Effect of biofield treatment on *Raoultella ornithinolytica* to distinguishing feature of the genotype.

Discussion

MDR emergence of *R. ornithinolytica* is a global health problem commonly associated with bacteremia, urinary tract infection, neonatal infections, and exist in underlying existing infection [6,26,27]. Increasing resistance in microorganism for antimicrobials becomes a major threat to health and economic problem which ultimately leads to allowing survival of the resistant bacteria and death of the susceptible ones. Major mechanistic pathways associated with resistant in microorganism are cell membrane alterations, which lead to decreased uptake of drug [28]; mutation occurs, that lead to over expression of drug target enzymes and the other common mechanism being the drug efflux pump [29]. Now-a-days, *R. ornithinolytica* acquired resistance against broad range of antimicrobials. Research study suggests that most of the clinical isolates of *R. ornithinolytica* were found resistant to all class of antimicrobials such as ampicillin, amoxicillin, cephalothin, cephotaxime, chloramphenicol, penicillin, gentamicin, rifampin, and streptomycin. Nitrofurantoin and meropenam showed highest sensitivity for *R. ornithinolytica* in all the clinical samples [30]. Our experimental control sample (*R. ornithinolytica*) showed similar sensitivity and resistant pattern of tested antimicrobials. Overall results showed increase in antimicrobial sensitivity after biofield treatment in 71.4% tested antimicrobials as compared to control. Mr. Trivedi's biofield treatment has significantly decreased the MIC values of 71.87% tested antimicrobials as compared to control. As an enteric pathogen, *R. ornithinolytica* causes enteric fever associated with syndrome like fever, headache, and abdominal pain. Best treatment therapy during the infection starts with amoxicillin/clavulanic acid for 10-14 days, but therapy depends upon the sensitivity pattern of clinical strain [31]. Different class of antimicrobials showed significant effect after biofield treatment viz. β -Lactam penicillins (amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin), cephalosporins (cefazolin, ceftriaxone, and cefuroxime), monobactam (azetronan), fluoroquinolones (ciprofloxacin and levofloxacin), aminoglycosides (amikacin and tobramycin) and tetracycline. Lucchetti et al. studied the effect of energy therapy as an alternate medicine on growth of bacterial culture, and found a significant effect [32]. Similarly, experimental results showed improved antimicrobial sensitivity and reduced MIC values suggest that biofield treatment could be a new alternative treatment approach to inhibit the growth of pathogenic bacteria.

Several phenotypic biochemical identification tests were available to differentiate the *Raoultella* species. Identification test of *R. ornithinolytica* was performed using a series of biochemical reactions

and the basic positive reactions were observed in case of urea, ornithine and lysine decarboxylase, citrate, glucose, and sucrose. It generally grown at 10°C with utilization of L-sorbose as a carbon source [33]. Experimental control group showed positive reaction in above general characteristic of *R. ornithinolytica*, but biofield treatment group showed a significant alteration i.e. negative reactions in biochemical such as cetrimide, cephalothin, kanamycin, ornithine and tobramycin. Further, these biochemical changes were studied by biotype number analysis which was performed using an automated system. A significant change in biotype number was found in treated group on day 10, and new organism was identified as *Klebsiella oxytoca* after biofield treatment as compared to control *R. ornithinolytica* (Table 4). Biofield treatment on pathogenic microorganism had been reported, which alter the biochemical reactions, followed by change biotype number and identification of new microorganism after treatment. Current results are well supported with recent reported study [34].

Overall results of antimicrobial assay suggest that Mr. Trivedi's biofield treatment has significantly improved the sensitivity and MIC value of most of the tested antimicrobials (Figure 1). Therefore, it may be possible that lower dose of antimicrobials might require with similar response after biofield treatment, which may minimize the side effects associated with higher doses of antimicrobials. Mr. Trivedi has the ability to harness energy from environment and altered the significant changes in microorganisms [15,16]. 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 alter that could lead to show different phenotypic characteristics. Biofield treatment might induce significant changes in MDR strain of *R. ornithinolytica*, so that tested antimicrobials were showed better susceptibility pattern, decreased MIC values, and altered biochemical reactions, against this microorganism.

Conclusion

Present study concludes that biofield treatment has the ability to inhibit the microbial growth, by significantly increasing the susceptibility pattern and decreasing the MIC values of 71% tested antimicrobials. Biofield treatment has significantly altered the biochemical reactions and biotype number of MDR strain of *R. ornithinolytica*. On the basis of changed biotype number after biofield treatment, new organism was identified as *Klebsiella oxytoca*. It is assumed that biofield treatment could be applied in biomedical health care system in future to improve the antimicrobial potency that enhance human well-being.

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