

Antimicrobial Susceptibility of *Proteus mirabilis*: Impact of Biofield Energy Treatment

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Abstract

Proteus mirabilis (*P. mirabilis*) is widespread in nature, mainly found in soil, water, and the flora of human gastrointestinal tract. The current study was attempted to investigate the effects of Mr. Trivedi's biofield energy treatment on *P. mirabilis* both in lyophilized as well as revived state for antimicrobial susceptibility, biochemical characteristics, and biotype. *P. mirabilis* cells were procured from MicroBioLogics Inc., USA, in a sealed pack bearing the American Type Culture Collection (ATCC 25933) number and stored according to the recommended storage protocol until needed for experiments. Two sets of ATCC samples were taken in this experiment and denoted as A and B. The ATCC A sample was revived and divided into two parts Gr.I (control) and Gr.II (revived); likewise, the ATCC B was labeled as Gr.III (lyophilized). Group II and III were given with biofield treatment. All experimental parameters were studied using automated MicroScan Walk-Away[®] system. The result of antimicrobial susceptibility and minimum inhibitory concentration showed 6.67% and 9.38% alteration, respectively in treated cells of *P. mirabilis* as compared to the control. In addition, the overall biochemical reactions were significantly altered (42.42%) in the treated groups with respect to the control. Moreover, biotype number was changed in the treated cells, Gr. II, day 5 (40061546) and day 10 (77365764), while without alteration of organism as compared to the control (40061544; *Proteus mirabilis*). The results suggested that biofield treatment has an impact on *P. mirabilis* in revived state predominately.

Keywords: *Proteus mirabilis*; Antimicrobial susceptibility; Biofield energy treatment; Biochemical reaction; Biotype

Abbreviations: NIH/NCCAM: National Institute of Health/National Center for Complementary and Alternative Medicine; ATCC: American Type Culture Collection; NBPC 30: Negative Breakpoint Combo 30; MIC: Minimum Inhibitory Concentration; UTIs: Urinary Tract Infections

Introduction

Proteus mirabilis (*P. mirabilis*) is a species of Gram-negative and facultative anaerobic bacteria that shows swarming motility and urease activity. *Proteus* ranked third as the cause of hospital-acquired infections [1]. The organism is rod-shaped and motile bacterium with diverse mode of transmission [2]. *P. mirabilis* is a common causative organism of urinary tract infection (UTI) in the complicated urinary tract, most frequently in patients with the indwelling catheters or structural abnormalities of the urinary tract [3]. It expresses various virulence factors which are involved in uropathogenesis like adhesins (i.e., PMP fimbriae), motility (i.e., flagella) toxins (i.e., hemolysin and *Proteus* toxic agglutinin), quorum-sensing (i.e., cell-cell communication), enzymes (i.e., urease) and immune invasion (i.e., metalloproteinase - ZapA) [4]. The infection is more prone to male than female [5]. In hospitals, it is the second most frequently isolated Enterobacteriaceae species after *Escherichia coli*. The wild-type isolates of this species are more susceptible to β -lactams antimicrobials [6]. The organism possess a black-brown colour pigment, which behaves like a melanin in many respects, such as solubility, bleaching by oxidizing agents and positive response to the Fontana-Masson assay [7]. Most types of antibiotics are sensitive to *P. mirabilis* such as penicillin's, cephalosporins, aminoglycosides, rifampin, fluoroquinolones, and phenicols while resistant to amoxicillin, cefotaxime and carbenicillin [8,9].

Therefore, an alternative strategy is needed against *P. mirabilis* infection. Biofield treatment has been known and popularized as an

alternative approach that may be useful for the assessment of sensitivity profile of the organism [10-12].

The human body can emit the electromagnetic waves in the form of bio-photons, which surrounds the body and it is commonly known as biofield. Therefore, the biofield can generate by moving electrically charged particles (ions, cell, molecule, etc.) inside the human body. According to Rivera-Ruiz et al., it was reported that electrocardiography has been extensively used to measure the biofield of human body [13]. Thus, human has the ability to harness the energy from environment or universe and can transmit into any living or nonliving object(s) around the Globe. The objects always receive the energy and responding into useful way that is called biofield energy and the process is known as biofield treatment. Biofield (putative energy fields) or electromagnetic based energy therapies, used to promote health and healing had been exclusively reported by National Institute of Health/National Center for Complementary and Alternative Medicine (NIH/NCCAM) [14]. Mr. Trivedi's unique biofield treatment (The Trivedi Effect[®]) has been known to transform the structural, physical and thermal properties of several metals and ceramic in materials science [15-17], improved the overall productivity of crops [18,19], and improved growth and anatomical characteristics of medicinal plants [20,21].

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Received December 21, 2015; **Accepted** January 28, 2016; **Published** February 04, 2016

Citation: Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, et al. (2016) Antimicrobial Susceptibility of *Proteus mirabilis*: Impact of Biofield Energy Treatment. J Microb Biochem Technol 8: 025-029. doi:10.4172/1948-5948.1000258

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Due to the clinical significance of this organism and literature reports on biofield treatment, the present work was undertaken to evaluate the impact of biofield treatment on *P. mirabilis* in relation to their antimicrobials susceptibility, biochemical reaction, and biotyping.

Materials and Method

P. mirabilis, American Type Culture Collection (ATCC 25933) strain was procured from MicroBioLogics, Inc., USA and stored with proper storage conditions until further use. All the tested antimicrobials and biochemicals were procured from Sigma-Aldrich (MA, USA). The antimicrobial susceptibility, biochemical reactions and biotype number were estimated with the help of MicroScan Walk-Away[®] (Dade Behring Inc., West Sacramento, CA, USA) using Negative Breakpoint Combo 30 (NBPC 30) panel with respect to control group (Gr.).

Study Design

Two ATCC 25933 samples A and B of *P. mirabilis* were grouped (Gr.). ATCC A sample was revived and divided into two parts named as Gr.I (control) and Gr.II (revived, treated); likewise, ATCC B was labeled as Gr.III (lyophilized, treated).

Biofield Treatment Strategy

Mr. Trivedi provided the biofield treatment through his energy transmission process to the treated groups without touching the samples. In this process energy is transferred to the absorbing medium (sealed microbial samples) and this may cause several changes to occur within the absorbing medium. The treated samples were assessed for the antimicrobial sensitivity, biochemical reactions, and biotyping as per experimental design. Whilst handing over these cultures to Mr. Trivedi for retreatment purposes, optimum precautions were taken to avoid contamination.

Antimicrobial Susceptibility Test

Investigation of antimicrobial susceptibility of *P. mirabilis* was carried out with the help of automated instrument, MicroScan Walk-Away[®] using NBPC 30 panel. The panel can be stored at 2 to 25°C for analysis. The panel was allowed to equilibrate to room temperature prior to rehydration. The tests carried out on MicroScan were miniaturized of the broth dilution susceptibility test that has been dehydrated. Briefly, the 0.1 mL of the standardized suspension of *P. mirabilis* was pipetted into 25 mL of inoculum water using pluronic and inverted 8 to 10 times and inoculated, rehydrated, and then subjected to incubation for 16 hours at 35°C. Rehydration and inoculation was performed using the RENOK[®] system with inoculators-D (B1013-4). 25 mL of standardized inoculum suspension was poured in to inoculum tray. The detailed experimental procedure and conditions were followed as per the manufacturer's instructions. MIC is defined as the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation. The antimicrobial susceptibility pattern (S: Susceptible, R: Resistant; and I: Intermediate) and minimum inhibitory concentration (MIC) values were determined by observing the lowest antimicrobial concentration showing inhibition of growth [22].

Biochemical Reaction Studies

Biochemical reactions of *P. mirabilis* were determined using MicroScan Walk-Away[®], system with NBPC 30 panel. The preparation of NBPC 30 panel, inoculum followed by dehydration and rehydration was performed similar way as mentioned in antimicrobial susceptibility assay for analysis of biochemical reaction followed by biotype number.

The detailed experimental procedures and conditions were followed as per the manufacturer's instructions [22].

Identification of Organism by Biotype Number

The biotype number of *P. mirabilis* was determined on MicroScan Walk-Away[®] processed panel data report with the help of biochemical reactions data [22].

Results and Discussion

Antimicrobial susceptibility test

The result of *P. mirabilis* susceptibility pattern and MIC values of tested antimicrobials after biofield energy treatment are summarized in Tables 1 and 2, respectively. The data were analyzed and compared with respect to the control (Gr. I). Study was carried out with thirty antimicrobials. Overall, the treated cells of *P. mirabilis* showed 6.67% alteration in antimicrobial sensitivity pattern as compared to the control. *P. mirabilis* was the common species causes majority of *Proteus* infections followed by *P. vulgaris*, and *P. penneri*. The *Proteus* isolates were generally susceptible to gentamicin, amikacin, ceftriaxone, cefuroxime and cefotaxime. The control data were well matched with literature [23]. The ampicillin sensitivity was converted from susceptible (S) to intermediate (I) with increase of MIC value by two-fold (≤ 8 to

S. No.	Antimicrobial	Type of Response			
		Gr. I	Gr. II		Gr. III (Day 10)
			Day 5	Day 10	
1.	Amikacin	S	S	S	S
2.	Amoxicillin/k-clavulanate	S	S	S	S
3.	Ampicillin/sulbactam	S	S	S	S
4.	Ampicillin	S	S	I	S
5.	Aztreonam	S	S	S	S
6.	Cefazolin	S	S	S	S
7.	Cefepime	S	S	S	S
8.	Cefotaxime	S	S	S	S
9.	Cefotetan	S	S	S	S
10.	Cefoxitin	S	S	S	S
11.	Ceftazidime	S	S	S	S
12.	Ceftriaxone	S	S	S	S
13.	Cefuroxime	S	S	S	S
14.	Cephalothin	S	I	S	S
15.	Chloramphenicol	S	S	S	S
16.	Ciprofloxacin	S	S	S	S
17.	Gatifloxacin	S	S	S	S
18.	Gentamicin	S	S	S	S
19.	Imipenem	S	S	S	S
20.	Levofloxacin	S	S	S	S
21.	Meropenem	S	S	S	S
22.	Moxifloxacin	S	S	S	S
23.	Nitrofurantoin	S	S	S	S
24.	Norfloxacin	S	S	S	S
25.	Piperacillin/Tazobactam	S	S	S	S
26.	Piperacillin	S	S	S	S
27.	Tetracycline	R	R	R	R
28.	Ticarcillin/k-clavulanate	S	S	S	S
29.	Tobramycin	S	S	S	S
30.	Trimethoprim/sulfamethoxazole	S	S	S	S

R: Resistant; S: Susceptible; I: Intermediate; Gr.: Group

Table 1: Antibiogram of *Proteus mirabilis*: Effect of biofield treatment on antimicrobial susceptibility.

S. No.	Antimicrobial	Type of Response			
		Gr. I	Gr. II		Gr. III (Day 10)
			Day 5	Day 10	
1.	Amikacin	≤ 16	≤ 16	≤ 16	≤ 16
2.	Amoxicillin/k-clavulanate	≤ 8/4	≤ 8/4	≤ 8/4	≤ 8/4
3.	Ampicillin/sulbactam	≤ 8/4	≤ 8/4	≤ 8/4	≤ 8/4
4.	Ampicillin	≤ 8	≤ 8	16	≤ 8
5.	Aztreonam	≤ 8	≤ 8	≤ 8	≤ 8
6.	Cefazolin	≤ 8	≤ 8	≤ 8	≤ 8
7.	Cefepime	≤ 8	≤ 8	≤ 8	≤ 8
8.	Cefotaxime	≤ 8	≤ 8	≤ 8	≤ 8
9.	Cefotetan	≤ 16	≤ 16	≤ 16	≤ 16
10.	Cefoxitin	≤ 8	≤ 8	≤ 8	≤ 8
11.	Ceftazidime	≤ 8	≤ 8	≤ 8	≤ 8
12.	Ceftriaxone	≤ 8	≤ 8	≤ 8	≤ 8
13.	Cefuroxime	≤ 4	≤ 4	≤ 4	≤ 4
14.	Cephalothin	≤ 8	16	≤ 8	≤ 8
15.	Chloramphenicol	≤ 8	≤ 8	≤ 8	≤ 8
16.	Ciprofloxacin	≤ 1	≤ 1	≤ 1	≤ 1
17.	ESBL-a Scrn	≤ 4	≤ 4	≤ 4	≤ 4
18.	ESBL-b Scrn	≤ 1	≤ 1	≤ 1	≤ 1
19.	Gatifloxacin	≤ 2	≤ 2	≤ 2	≤ 2
20.	Gentamicin	≤ 4	≤ 4	≤ 4	≤ 4
21.	Imipenem	≤ 4	≤ 4	≤ 4	≤ 4
22.	Levofloxacin	≤ 2	≤ 2	≤ 2	≤ 2
23.	Meropenem	≤ 4	≤ 4	≤ 4	≤ 4
24.	Moxifloxacin	≤ 2	≤ 2	≤ 2	≤ 2
25.	Nitrofurantoin	64	64	>64	64
26.	Norfloxacin	≤ 4	≤ 4	≤ 4	≤ 4
27.	Piperacillin/tazobactam	≤ 16	≤ 16	≤ 16	≤ 16
28.	Piperacillin	≤ 16	≤ 16	≤ 16	≤ 16
29.	Tetracycline	>8	>8	>8	>8
30.	Ticarcillin/k-clavulanate	≤ 16	≤ 16	≤ 16	≤ 16
31.	Tobramycin	≤ 4	≤ 4	≤ 4	≤ 4
32.	Trimethoprim/sulfamethoxazole	≤ 2/38	≤ 2/38	≤ 2/38	≤ 2/38

MIC data are presented in µg/mL; Gr.: Group; ESBL: Extended spectrum β-lactamase

Table 2: Effect of biofield treatment on *Proteus mirabilis* to minimum inhibitory concentration (MIC) value of tested antimicrobials.

16 µg/mL) in Gr. II on day 10 as compared to the untreated group. In additionally, cephalothin was converted from S to I with increase of MIC value by two-fold (≤ 8 to 16 µg/mL) in Gr. II on day 5 as compared to the Gr. I. The decrease susceptibility and increase MIC value were well supported with literature [24]. The susceptibility and MIC data of both antibiotics ampicillin and cephalothin were well correlated in this experiment. The MIC value of nitrofurantoin was slightly increased in Gr. II on day 10 as compared to the control. Overall, the treated cells of *P. mirabilis* showed 9.38% alteration in MIC values of tested antimicrobials. Rest of the antimicrobials did not show any alteration in terms of antimicrobial susceptibility and MIC values as compared to control.

Biochemical reactions studies

The study of biochemical reactions can be utilized to identify the enzymatic and metabolic characteristic features of microbes. Microorganisms can be categorically differentiated based on their utilization of specific biochemicals as nutrients during the process of metabolism or enzymatic reactions. Table 3 shows the conventional biochemical tests necessary for the differentiation of *P. mirabilis*. The

S. No.	Code	Biochemical	Gr. I	Type of Response		
				Gr. II		Gr. III (Day 10)
				Day 5	Day 10	
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.	H ₂ S	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/glucose	+	+	+	+
21.	ONPG	Galactosidase	-	-	-	-
22.	ORN	Ornithine	+	+	+	+
23.	OXI	Oxidase	-	-	-	-
24.	P4	Penicillin	+	+	+	-
25.	RAF	Raffinose	-	-	+	-
26.	RHA	Rhamnose	-	-	+	-
27.	SOR	Sorbitol	-	-	+	-
28.	SUC	Sucrose	-	-	+	-
29.	TAR	Tartrate	-	-	-	-
30.	TDA	Tryptophan deaminase	+	+	+	+
31.	TO4	Tobramycin	-	-	-	-
32.	URE	Urea	+	+	+	+
33.	VP	Voges-Proskauer	+	+	+	+

-, (negative); +, (positive); Gr.: Group

Table 3: Effect of biofield treatment on *Proteus mirabilis* to the biochemical reaction pattern.

tested biochemicals such as adonitol (ADO), arabinose (ARA), esculin hydrolysis (ESC), nitrofurantoin (FD64), kanamycin (K4), lysine (LYS), malonate (MAL), melibiose (MEL), raffinose (RAF), rhamnose (RHA), sorbitol (SOR), and sucrose (SUC) were converted from negative (-) to positive (+) reaction in revived treated group (Gr. II) on day 10, as compared to the control. However, it did not show any change in others groups. Cephalothin was converted from negative (-) to positive (+) reaction in revived treated group (Gr. II) on day 5, while did not produce any effect on others treated samples as compared to the control. Moreover, penicillin showed positive (+) reaction in Gr. II (both days 5 and 10), while did not show any response in lyophilized treated group (Gr. III) as compared to the control (i.e., negative reaction). Overall, the treated cells of *P. mirabilis* showed 42.42% alteration in biochemical reaction pattern of tested antimicrobials. Based on literature there was two types of reaction phenomenon of indole, i.e., indole positive *P. mirabilis* and indole negative *P. mirabilis* [25]. In this experiment based on the findings of biochemical reaction it is assumed that the strain is indole negative. Rest of the biochemicals did not show any alteration of biochemical reactions in all the treated groups as compared to the control (Table 3).

Identification of organism by biotype number

The species (*P. mirabilis*) was identified based on variety of conventional biochemical characters and biotyping. Biotype number of particular organism was evaluated after interpreting the results of the biochemical reactions. The biotype number then led to the particular organism identification. In this experiment, biotyping was performed using automated systems, and results showed significant change in biotype number (77365764) in the biofield treated Gr. II (on day 10) as compared to control Gr. I (40061544). Although, the organism was not altered after biofield energy treatment. The biotype number was also changed in Gr. II on day 5 (40061546) as compared to the control. Rest of the group (Gr. III) did not show any alteration of biotype number after biofield energy treatment as compared to their respective control (Table 4).

Biofield treatment might be responsible for alteration in microorganism at enzymatic and/or genetic level, which may act on receptor protein. While altering receptor protein, ligand-receptor/protein interactions may altered that could lead to show different phenotypic characteristics [26]. Biofield treatment might induce significant changes in revived strain of *P. mirabilis* and altered antimicrobials susceptibility pattern, MIC values, biochemical reactions, which ultimately change the biotype number of microorganism. As a result, the microbe that was susceptible to a particular antimicrobial in control sample now converted into intermediate in the treated cells of *P. mirabilis* predominately after biofield energy treatment.

In this experiment, the main objective was to see the impact of Mr. Trivedi's biofield energy treatment on an opportunistic hospital acquired pathogen of *P. mirabilis* in *in vitro*. Based on the findings of antimicrobial susceptibility pattern of ampicillin and cephalothin showed that the susceptible nature of both control samples and it became intermediate on Gr. II. So far our group had been published many research articles regarding the effect biofield treatment on ATCC and multidrug resistant (MDR) strains [17-19]. Based on these results, it is expected that biofield treatment has the scope to be an alternative approach than the existing antimicrobial therapy in near future.

Conclusion

Altogether, the biofield treatment showed 6.67% alteration in antimicrobial susceptibility pattern with 9.38% change in MIC values of tested antimicrobials against the strain of *P. mirabilis*. It also significantly altered the biochemical reactions pattern (42.42%) and biotype number of biofield treated strain of *P. mirabilis*. The biotype number was changed in the treated group II on day 5 (40061546) and on day 10 (77365764) as compare to the control (40061544) without alteration of microbes. Thus, Mr. Trivedi's unique biofield energy treatment could be applied as alternative therapeutic approach against antimicrobials in future.

Feature	Gr. I (Control)	Gr. II		Gr. III (Day 10)
		Day 5	Day 10	
Biotype	40061544	40061546	77365764	40061544
Organism Identification	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i>	<i>Proteus mirabilis</i> (Very rare biotype)	<i>Proteus mirabilis</i>

Gr.: Group

Table 4: Effect of biofield treatment on biotype number of *Proteus mirabilis*.

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