

Effect of Biofield Treatment on Antimicrobials Susceptibility Pattern of *Acinetobacter baumannii* - An Experimental Study

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Abstract

Global emergence of *Acinetobacter baumannii* (*A. baumannii*) displays a mechanism of resistance to all existing antimicrobials. Objective of this study was to investigate the effect of biofield treatment on antimicrobial sensitivity pattern, minimum inhibitory concentration (MIC), biochemical reactions and biotype number of *A. baumannii*. *A. baumannii* cells were procured from MicroBioLogics in sealed packs bearing the American Type Culture Collection (ATCC 19606) number and stored according to the recommended storage protocols until needed for experiments. Two sets of ATCC samples were taken in this experiment and denoted as A and B. ATCC-A sample was revived and divided into two parts i.e. Gr.I (control) and Gr.II (revived) analyzed on day 5 and 10, respectively; likewise, ATCC-B was labeled as Gr.III (lyophilized) and was assessed on day 10. Gr.II and III were treated with Mr. Trivedi's biofield and were analyzed for its antimicrobial sensitivity, MIC value, biochemical reactions and biotype number with respect to control. Experimental results showed the impact of biofield treatment directly onto the revived and lyophilized form of *A. baumannii* and found alteration both in qualitative and quantitative aspect as compared with untreated groups. These results showed altered sensitivity pattern of antimicrobials in biofield treated group as compared to control. Apart from altered MIC values, changes were also observed in biotype number of revived treated group as compared to control. These findings suggest that biofield treatment can prevent the emergence of absolute resistance of existing antimicrobials to *A. baumannii*.

Keywords: *Acinetobacter baumannii*; Antimicrobials susceptibility; Biofield treatment; Biochemical reactions; Biotyping

Introduction

Acinetobacter baumannii is a Gram-negative, obligate aerobic coccus mostly causing infections in healthcare settings and found mostly in soil, water, and sewage [1]. Bacteremia caused by *A. baumannii* is a common nosocomial infection in critically ill patients. *Acinetobacter* spp. consists of non-fermenting, coccobacilli and they are opportunistic pathogens [2]. Initially, *A. baumannii* infections were at low potential but now a days it is responsible for several types of hospital acquired infections and regarded as one of the most troublesome pathogens [3,4]. Unique feature of this organism is the ability to utilize different carbon sources and its ability to survive in a range of temperatures and pH conditions. La Scola and Raoult [5] studied and found that this microbe may utilize the arthropod source for mean of transmission, which they isolated from human body lice. Mortality rates of patients suffering *A. baumannii* infections can be as high as 75% [6]. Currently, no alternative treatment approaches are available for multidrug resistance microorganism, but biofield treatment may be a new approach to cope up with this global problem.

Biofield is the name given to the electromagnetic field that permeates and surrounds living organisms. It is the scientifically preferred term for the biologically produced electromagnetic and subtle energy field that provides regulatory and communication functions within the organism. However, the energy can exist in several forms such as kinetic, potential, electrical, magnetic, and

nuclear. 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 that is called biofield energy and the process is known as biofield treatment. In spite of countless study reports of the effectiveness of biofield therapies [7], there are very few well controlled and peer-reviewed experimental studies [8]. According to law of mass-energy inter-conversion [9], the conversion of mass into energy is well stabilized, but its inversion i.e. energy into mass has not yet proved scientifically. Whenever these electrical signals fluctuate with time, the magnetic field generates as per the Ampere-Maxwell law, and cumulatively known as electromagnetic field. As responses by humans can be accounted for by the placebo effect, these experiments on lower organisms were designed in order to directly test the impact of biofield energy through scientific studies to rule out the placebo effect. It is widely accepted that lyophilization is the method most commonly used to store and transport microbial cultures, as change in the biochemical and enzymatic characteristics of an organism cannot be carried out in this state. Mr. Trivedi's biofield treatment (The Trivedi effect[®]) is well-known to change the various physicochemical characteristics of various materials after his biofield treatment. In addition, Mr. Trivedi biofield has considerably altered the antimicrobials susceptibility and biochemical reactions of microbes against tested antimicrobials [10-12]. It has also significantly altered the crystalline and powder characteristics of metals [13-20]. In agriculture, biofield treated crops has been reported for a significant change on growth, characteristics and yield of plants [21-25]. There are

scanty reports on investigating biofield treatment against microbes. Present study reports the impact of biofield on *A. baumannii*, for its antimicrobials susceptibility pattern along with biochemical properties of revived and lyophilized cells of *A. baumannii* as compared to its control groups.

Experimental Section

A. baumannii strains were procured from MicroBioLogics, Inc., USA, in two sets A and B. Two different sealed packs were stored with proper storage conditions until further use. Following parameters like antimicrobial susceptibility, biochemical reactions and biotype number were estimated with help of automation on the Microscan Walkaway system (Dade Behring Siemens) using NBPC30 panel with respect to control groups. Finally, all the groups (i.e. control and treated) were investigated for its antimicrobial susceptibility, biochemical reactions pattern and biotyping. All antimicrobials and biochemicals were procured from Sigma Aldrich, USA.

Study Design and Biofield Treatment Modality

Two ATCC (American Type Culture Collection) samples (ATCC A and B) of *A. baumannii* were grouped and subject to biofield treatment. ATCC A sample was revived and divided into two parts Gr.I (control) and Gr.II (revived); likewise, ATCC B was labeled as Gr.III (lyophilized). Then group II and III were treated with Mr. Trivedi's biofield energy. The treatment groups were in sealed pack and handed over to Mr. Trivedi for biofield treatment under laboratory condition. Mr. Trivedi provided the treatment through his energy transmission process to the treated groups without touching the samples. Gr.II was assessed on the 5th and 10th days after treatment while Gr. III was assessed on 10th day. Finally, all the groups (control and treated) were investigated for antimicrobial susceptibility, biochemical reactions pattern and biotyping compared with control.

Antimicrobial Susceptibility Assay

Antimicrobial susceptibility pattern of *A. baumannii* was studied using MicroScan Walk-Away[®] NBPC30 as per manufacturer's instructions. The qualitative antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate, NR: Not Reported, and R: Resistant) and minimum inhibitory concentration (MIC) were determined by observing the change in antimicrobial concentration as per the latest CLSI guidelines [26].

Biochemical Reaction and Biotype Number Studies

The biochemical reactions and biotype number of *A. baumannii* were also determined by MicroScan Walk-Away[®] system. Effect of biofield treatment on *A. baumannii* to the vital processes occurring in living organisms were studied [27].

S. No.	Antimicrobial	Type of Response			
		Gr.I		Gr.III	
		Control	Day 5	Day 10	Day 10
1	Amikacin	S	S	S	S
2	Amoxicillin/K-clavulanate	NR	NR	NR	NR
3	Ampicillin/sulbactam	S	S	S	S

4	Aztreonam	I	I	I	I
5	Cefepime	I	I	I	S
6	Cefotaxime	I	I	I	I
7	Ceftazidime	S	S	S	S
8	Ceftriaxone	I	I	I	I
9	Chloramphenicol	R	R	R	R
10	Ciprofloxacin	S	S	S	S
11	Gentamicin	I	R	R	R
12	Imipenem	S	S	S	S
13	Levofloxacin	S	S	S	S
14	Meropenem	S	S	S	S
15	Piperacillin	S	S	S	I
16	Tetracycline	S	S	S	S
17	Ticarcillin/K-clavulanate	S	S	S	S
18	Tobramycin	S	S	S	S
19	Trimethoprim/sulfamethoxazole	R	R	R	R

R: Resistant; I: Intermediate; S: Susceptible; NR: Not Reported; Gr: Group

Table 1: Antimicrobial susceptibility assay of control and biofield treated *A. baumannii*.

Results

Antimicrobials susceptibility assay

The data pertaining to the antimicrobials susceptibility tests and details of MIC values of biofield effect were observed, analyzed and reported in Tables 1 and 2. The effect of biofield treatment showed that the cefepime, converted from I → S in lyophilized treated group, gentamicin converted from I → R in all the treated group and piperacillin converted from S → I only in lyophilized biofield treatment group as compared to control. Quantitative assessment, MIC results showed that amoxicillin/K-clavulante, β-lactamase-inhibiting antimicrobial have showed a decrease in values i.e. from 16/8 to less than 8/4 µg/mL in both the treated groups as compared with control. Gentamicin value increased in all the groups after biofield treatment as compared to control. Nitrofurantoin showed increased MIC value in Gr. II, while norfloxacin showed increased MIC in Gr. II as compared to control. Rest antimicrobials did not show any change in susceptibility pattern and MIC value after biofield treatment compared to control.

S. No.	Antimicrobial	Type of Response			
		Gr.I		Gr.III	
		Control	Day 5	Day 10	Day 10
1	Amikacin	≤ 16	≤ 16	≤ 16	≤ 16
2	Amoxicillin/K-clavulanate	16/8	≤ 8/4	≤ 8/4	≤ 8/4

3	Ampicillin/Sulbactam	≤ 8/4	≤ 8/4	≤ 8/4	≤ 8/4
4	Ampicillin	>16	>16	>16	>16
5	Aztreonam	16	16	16	16
6	Cefazolin	>16	>16	>16	>16
7	Cefepime	16	16	16	16
8	Cefotaxime	32	32	32	32
9	Cefotetan	>32	>32	>32	>32
10	Cefoxitin	>16	>16	>16	>16
11	Ceftazidime	≤ 8	≤ 8	≤ 8	≤ 8
12	Ceftriaxone	32	32	32	32
13	Cefuroxime	>16	>16	>16	>16
14	Cephalothin	>16	>16	>16	>16
15	Chloramphenicol	>16	>16	>16	>16
16	Ciprofloxacin	≤ 1	≤ 1	≤ 1	≤ 1
17	ESBL-a Scrm	>4	>4	>4	>4
18	ESBL-b Scrm	>1	>1	>1	>1
19	Gatifloxacin	≤2	≤2	≤2	≤2
20	Gentamicin	8	>8	>8	>8
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	8	8	8	>8
27	Piperacillin	≤ 16	≤ 16	≤ 16	64
28	Tetracycline	≤ 4	≤ 4	≤ 4	≤ 4
29	Ticarcillin/K-clavulanate	≤ 16	≤ 16	≤ 16	≤ 16
30	Tobramycin	≤ 4	≤ 4	≤ 4	≤ 4
31	Trimethoprim/sulfamethoxazole	>2/38	>2/38	>2/38	>2/38

MIC data are presented in µg/mL; ESBL-a,b Scrm: Extended-spectrum-beta-lactamase screen; Gr: Group

Table 2: Minimum inhibitory concentration (MIC) of *A. baumannii* for tested antimicrobials.

Identification by biochemical reaction

Biochemical reactions denoted with codes were tabulated and showed positive reaction in case of nitrofurantoin on day 5 and 10 i.e. from negative (-) to positive (+) in revived treated group (Gr II) with respect to control (Table 3).

S. No.	Code	Biochemical	Type of Response			
			Gr.I	Gr.II		Gr.III
			Control	Day 5	Day 10	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	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	-	-	-	-

-. negative; +. positive; Gr: Group

Table 3: Effect of biofield treatment on biochemical reactions of *A. baumannii*.

Identification by biotype number

Biochemical tests results revealed a change in biotype number in Gr.II at day 5 and 10 of *A. baumannii* after biofield treatment with respect to control (Table 4).

Feature	Gr.I	Gr.II		Gr.III
	Control	Day 5	Day 10	Day 10
Biotype number	60700	60720	60720	60700
Organism Identification	<i>A. baumannii</i>	<i>A. baumannii</i>	<i>A. baumannii</i>	<i>A. baumannii</i>
Gr: Group				

Table 4: Effect of biofield treatment on *A. baumannii* to distinguishing feature of the genotype.

Discussion

Biofield treatment is effective and has been experimentally demonstrated change in susceptibility pattern, biochemical reaction and biotype number in experimental setup designed above. Results were unexpected and unprecedented based on the literature so far. These results assumed the presence of energy in biofield with which microbe reacts, which led to alteration of phenotypic characteristics of the microorganism.

Due to the high frequency of antimicrobial multidrug resistance among clinical isolates of *A. baumannii*, it causes serious problems in the choice of appropriate antimicrobials in the past 15 years [3]. This experiment showed that, biofield treatment induces changes in susceptibility pattern of cefepime, gentamicin and piperacillin antimicrobials. However, most frequent mechanism of resistance expressed by Gram-negative organisms against cefepime is the production of β -lactamases that are able to hydrolyze the drugs [28]. Cefepime resistance mechanism is demonstrated in *A. baumannii* by a combination of hyperproduction of the chromosomal OXA-51/69-like carbapenemases, activation of efflux pumps (i.e. AdeABC) and probably porin changes [29]. The expression of OXA-type carbapenemases in the absence of the other mechanisms of resistance does not result in high-level cefepime resistance. Similarly, expression of chromosomal *Acinetobacter*-derived cephalosporinase by *A. baumannii* does not seem to confer resistance. By contrast, production of certain acquired class D OXA-type carbapenemases and/or metallo- β -lactamases are important mechanisms of resistance against cefepime [30,31]. Change in susceptibility pattern in cefepime might be due to the influence of biofield treatment, which may inhibits the production of beta-lactamases enzymes (carbapenemases like) or inactivation of efflux pumps. Quantitative aspect showed lowering in MIC values and change in biotype number which was an accurate approach for epidemiologic investigation of *A. baumannii* as compared with untreated group. Biotype number of particular organism was arrived at after interpreting the results of the biochemical reactions which led to the particular organism identification. Biotyping makes use of the pattern of metabolic activities expressed by an isolate, colonial

morphology and environmental tolerances. In this experiment, biotyping was performed using automated system and found a significant change in the biofield treated Gr. II on day 5 and 10. Among the β -lactamase antimicrobials and inhibitors, amoxicillin/clavulanic acid have shown good efficacy in treating *A. baumannii* infections [32] and their decreases MIC values may represent an alternative treatment option for severe nosocomial infections caused due to *A. baumannii*. The biochemical tests like oxidases, nitrate, indole negative and catalase positive are the basic biochemical characteristics of *A. baumannii* confirmed in this experiment, as *Acinetobacter* spp. and members of the Moraxellaceae family [2]. In addition, it should be noted that different bacterial strains differ in their characteristics, and it is possible that some microbes are more susceptible/resistant towards biofield treatment than others. The present research data suggest that biofield treatment changed the susceptibility of antimicrobials against microbe, in a continuation of already published reports [10-25]. As a result, the microbe that was intermediate responsiveness changed to either resistant or susceptible to a particular drug after biofield treatment.

Conclusion

Present study concludes the impact of biofield treatment on changes in susceptibility pattern of *A. baumannii* which was confirmed by standard procedures with respect to MIC value, biochemical study and biotype number. Biofield treatment could be applied to alter the sensitivity of antimicrobials to fight against infections microbes due to the emergence of multi drug-resistant strains.

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