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Antibiogram Typing of Biofield Treated Multidrug Resistant Strains of *Staphylococcus* Species

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Abstract: Antimicrobial resistance is a global health issue in the developing countries. This study was carried out to evaluate the impact of Mr. Trivedi's biofield energy treatment on multidrug resistant (MDR) clinical lab isolates (LSs) of *Staphylococcus* species viz. *Staphylococcus haemolyticus* (LS 18), *Staphylococcus epidermidis* (LS 21), and *Staphylococcus aureus* (LS 30). Each strain was divided into the two groups i.e. control and treated. The control and treated groups were analyzed for the antimicrobial susceptibility pattern, minimum inhibitory concentration (MIC), biochemical analysis and biotype number using MicroScan Walk-Away[®] system. The analysis was done on day 10 after biofield treatment and compared with the control group. The sensitivity of erythromycin was improved from resistant to susceptible, while levofloxacin sensitivity was also improved from intermediate to susceptible in LS 21 isolate. The MIC results showed a decrease in the concentrations of ceftriaxone, erythromycin, imipenem, and levofloxacin antimicrobials in LS 21 as compared to the control. Linezolid and vancomycin also showed decrease in MIC as compared to the control in LS 30. Overall, 20.69% antimicrobials showed decrease in MIC value out of the tested twenty-nine after biofield treatment in *Staphylococcus* species. The biochemical study showed a 25% alteration in biochemical reactions as compared to the control. A significant change was reported in biotype numbers for all the three strains of MDR *Staphylococcus* species after biofield treatment as compared to the respective control group. On the basis of changed biotype number (306366) after biofield treatment in LS 18, the new organism was identified as *Staphylococcus simulans* with respect to the control species i.e. *Staphylococcus haemolyticus* (302302). The control group of *S. epidermidis* and *S. aureus* showed biotype number as 303064 and 757153 respectively. After biofield treatment, LS 21 and LS 30 isolates showed altered biotype number as 307064 and 317153 respectively. Overall, results conclude that biofield treatment could be used as complementary and alternative treatment strategy against multidrug resistant strains of *Staphylococcus* species with improved sensitivity and reduced MIC values of antimicrobial.

Keywords: *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, Biofield Energy Treatment, Multidrug-Resistant, Antibiogram, Biotyping

1. Introduction

Staphylococci are members of the family *Staphylococcaceae*. They are Gram-positive, catalase-positive and appear as isolated or in irregular grape-like clusters. The classification in this family is based on the production of coagulase [1]. The genus *Staphylococcus* represents a broad family of species and subspecies, which are widely distributed in nature and consists of approximately 45 species. The skin and mucosal membranes are the main

habitats of the human infections. Staphylococci usually maintain the symbiotic relationship with host followed by crossing the cutaneous barrier and may reach other tissues and proliferate [2]. *Staphylococcus haemolyticus* (*S. haemolyticus*) is the second most common coagulase-negative staphylococci in the human blood, and is associated with bacteremia, skin or soft tissue infections, prosthetic joint infections, or meningitis [3]. *S. haemolyticus* and *Staphylococcus lugdunensis* recently reported to have association with native valve endocarditis [4].

Staphylococcus epidermidis (*S. epidermidis*) being the most prevalent species on the skin causing different central line-associated bloodstream infections [5]. *S. epidermidis* is regarded as the commensal microorganism on the human skin which is mostly associated with the nosocomial infections [6]. *Staphylococcus aureus* (*S. aureus*) is classified as the major serious human pathogen causing infections worldwide, however the other strains of *Staphylococcus* coagulase negative cocci have recently reported to the cause serious human infections [7]. Ten major and basic causes of death worldwide are due to the nosocomial blood infections by pathogenic clinical isolates of *S. aureus* [8]. *S. aureus* produces toxins that cause various disturbances of the immune system [9]. Increased resistance among the different pathogenic strains of *Staphylococcus* species against broad spectrum antibiotics is the major health problem, which limits the treatment options in front of the physicians. Alternative treatment approach to alter the sensitivity profile of antimicrobials using biofield treatment on multidrug resistant (MDR) microorganism is recently reported [10, 11].

Biofield is the name given to the electromagnetic field that permeates and surrounds the 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. In spite of several study reports of the effectiveness of the biofield healing therapies [12], there are very few well-controlled and peer-reviewed experimental studies on pathogenic microorganisms. The biofield energy can be monitored by using electromyography (EMG), electrocardiography (ECG) and electroencephalogram (EEG) [13]. 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 Mr. Trivedi's unique biofield energy treatment is also known as The Trivedi Effect[®]. Mr. Trivedi's biofield treatment was extensively studied in different fields such as in material science [14-16], agricultural science [17-19], and in biotechnology [20]. Further, the biofield treatment has considerably altered the susceptibility of antimicrobials and biotype of microbes [10, 11, 21]. By considering the significant reports on the biofield treatment, the present work was designed to evaluate the influence of biofield energy treatment on MDR strains of *Staphylococcus* species (*S. haemolyticus*, *S. epidermidis* and *S. aureus*) with respect to antibiogram typing, biochemical reaction pattern, followed by biotyping.

2. Materials and Methods

2.1. Experimental Design and Bacterial Isolates

MDR clinical lab isolates (LSs) of *S. haemolyticus* (LS 18), *S. epidermidis* (LS 21) and *S. aureus* (LS 30) were obtained from stored stock cultures in Microbiology Lab, Hinduja Hospital, Mumbai. Each MDR strains of

Staphylococcus species was divided into two groups *i.e.* control and treatment.

2.2. 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 following parameters like antimicrobial susceptibility, minimum inhibitory concentration (MIC), biochemical reactions, and biotype number were measured using MicroScan Walk-Away[®] automated system (Dade Behring Inc., USA). All antimicrobials and biochemicals were procured from Sigma Aldrich, USA.

2.3. Evaluation of Antimicrobial Susceptibility Assay

The tests carried out on MicroScan were miniaturized of the broth dilution susceptibility tests that have been dehydrated. Briefly, the standardized suspensions of each strain were inoculated, rehydrated, and then subjected to incubation for 16 hour at 35°C. The detailed experimental procedures and conditions were followed as per the manufacturer's instructions. Antimicrobial susceptibility patterns of MDR strains of *Staphylococcus* species (LS 18, LS 21, and LS 30) were studied using MicroScan Walk-Away[®] using (Dade Behring Inc., West Sacramento, CA, USA) using Positive Breakpoint Combo 20 (PBPC 20) panel as per the clinical and laboratory standards institute (CLSI) guidelines. The antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate, R: Resistant, and BLAC: β -lactamase positive) and MIC values were determined by observing the lowest antimicrobial concentration showing growth inhibition [22].

2.4. Biochemical Study

Biochemical studies of each MDR strains of *Staphylococcus* species were determined by MicroScan Walk-Away[®] using PBPC 20 panel system, it interprets the microbe biochemical results with the use of a photometric or fluorogenic reader. On the basis of nature of bacilli (Gram-negative or Gram-positive), it generates computerized reports using conventional panels, which utilizes the photometric reader and provide identification results. Before commencing the experiment, the NBPC 30 panel was first incubated and read on the MicroScan Walkaway system. After completion of reading on the Walkaway system, the PBPC 20 panel was removed from system and read on the Biomic system within 1 hour. MicroScan Walk-Away instrument consist of a database associated with collective information, which was required to identify the group, genera, or species of the family. Detailed experimental procedure was followed as per manufacturer-recommended instructions. Biochemicals used in the study were arabinose, arginine, bacillosamine, bile

esculin, β -lactamases, crystal violet, indoxyl phosphatase, inulin, acidification lactose, mannitol, mannose, micrococcus screen, sodium chloride, nitrate, novobiocin, optochin, p-nitro phenyl β -D-glucuronide, p-nitro phenyl β -D-galactopyranoside, phosphatase, pyruvate, pyridonol arylamidase, raffinose, rambrose, sorbitol, thymidine free growth, acidification trehalose, urea, and Voges-Proskauer [22].

2.5. Identification by Biotype Number

The biotype number of each MDR strains of *Staphylococcus* species in control and treated sample was determined followed by identification of microorganism by MicroScan Walk-Away[®] processed panel data report with the help of biochemical reaction data [22].

3. Results and Discussion

3.1. Antimicrobial Susceptibility Study

Antimicrobial sensitivity pattern and MIC values of control and biofield treated MDR strains of *Staphylococcus* species are summarized in Table 1 and 2, respectively. Altered sensitivity of tested antimicrobials were observed on day 10 after biofield treatment, and compared with respect to the control. Antimicrobial sensitivity after biofield treatment in case of *S. haemolyticus* (LS 18) strain showed no change in twenty-seven tested antimicrobials with respect to the control. Erythromycin sensitivity was improved *i.e.* resistant

to susceptible, while levofloxacin sensitivity was also improved from intermediate to susceptible in biofield treated *S. epidermidis* (LS 21). Now, erythromycin and levofloxacin are active against biofield treated *S. epidermidis*. Most of the isolates of *S. epidermidis* had been observed for multidrug resistant against penicillin, oxacillin, gentamycin, erythromycin, and doxycycline. Resistant pattern of most of the antimicrobials in control group is well supported with literature data [23]. Rest of the tested antimicrobials did not show any alteration in antimicrobial sensitivity in LS 18, LS 21 and LS 30. MIC results showed an alteration of 20.69% among twenty-nine tested antimicrobials. The MIC value of erythromycin after biofield treatment on *S. epidermidis* showed eight-fold decrease (*i.e.* >4 to ≤ 0.5 $\mu\text{g/mL}$) in concentration required to inhibit the visible growth of microorganism with respect to the control. Ceftriaxone also showed four fold decrease in MIC value after biofield treatment in LS 21 with respect to control *i.e.* 32 to ≤ 8 $\mu\text{g/mL}$. Imipenem (>8 to ≤ 4 $\mu\text{g/mL}$) and levofloxacin (4 to ≤ 2 $\mu\text{g/mL}$) showed two-folds decrease in MIC value in biofield treated LS 21 as compared to control. Biofield treatment in *S. aureus* (LS 30) also showed two-fold decrease in MIC value in case of linezolid (>4 to ≤ 2 $\mu\text{g/mL}$) and vancomycin (4 to ≤ 2 $\mu\text{g/mL}$) as compared to respective control. Remaining antimicrobials did not show any alteration in MIC values in LS 21 and LS 30. *Staphylococcus haemolyticus* (LS 18) did not show any alteration with respect to antimicrobial susceptibility pattern and MIC value among tested antimicrobials (Table 2).

Table 1. Effect of biofield treatment on multidrug resistant lab isolates of *Staphylococcus* spp. to antimicrobial susceptibility.

S. No.	Antimicrobial	LS 18		LS 21		LS 30	
		C	T	C	T	C	T
1.	Amoxicillin/k-clavulanate	R	R	R	R	R	R
2.	Ampicillin/sulbactam	R	R	R	R	R	R
3.	Ampicillin	BLAC	BLAC	BLAC	BLAC	BLAC	BLAC
4.	Azithromycin	R	R	R	R	R	R
5.	Cefazolin	R	R	R	R	R	R
6.	Cefepime	R	R	R	R	R	R
7.	Cefotaxime	R	R	R	R	R	R
8.	Ceftriaxone	R	R	R	R	R	R
9.	Cephalothin	R	R	R	R	R	R
10.	Chloramphenicol	S	S	S	S	R	R
11.	Ciprofloxacin	R	R	R	R	R	R
12.	Clindamycin	R	R	R	R	R	R
13.	Erythromycin	R	R	R	S	R	R
14.	Gatifloxacin	I	I	S	S	R	R
15.	Gentamicin	R	R	R	R	R	R
16.	Imipenem	R	R	R	R	R	R
17.	Levofloxacin	R	R	I	S	R	R
18.	Linezolid	S	S	S	S	-	S
19.	Moxifloxacin	S	S	S	S	R	R
20.	Ofloxacin	R	R	I	I	R	R
21.	Oxacillin	R	R	R	R	R	R
22.	Penicillin	BLAC	BLAC	BLAC	BLAC	BLAC	BLAC
23.	Rifampin	R	R	R	R	R	R
24.	Synercid	S	S	S	S	S	S
25.	Tetracycline	R	R	S	S	R	R
26.	Trimethoprim/sulfamethoxazole	R	R	R	R	R	R
27.	Vancomycin	S	S	S	S	S	S

C: Control; T: Treatment; LS: Lab Isolate; R: Resistant; I: Intermediate; S: Susceptible; LS 18: *S. haemolyticus*; LS 21: *S. epidermidis*; LS 30: *S. aureus*; BLAC: β -lactamase positive; -: Not tested.

Table 2. Minimum inhibitory concentration (MIC) of tested antimicrobials for multidrug resistant lab isolates of *Staphylococcus* spp.

S. No.	Antimicrobial	LS 18		LS 21		LS 30	
		C	T	C	T	C	T
1.	Amoxicillin/k-clavulanate	>4/2	>4/2	≤4/2	≤4/2	>4/2	>4/2
2.	Ampicillin/sulbactam	16/8	16/8	≤8/4	≤8/4	16/8	16/8
3.	Ampicillin	>8	>8	8	8	>8	>8
4.	Azithromycin	>4	>4	>4	>4	>4	>4
5.	Cefazolin	>16	>16	≤8	≤8	>16	>16
6.	Cefepime	>16	>16	≤8	≤8	>16	>16
7.	Cefotaxime	>32	>32	≤8	≤8	>32	>32
8.	Ceftriaxone	>32	>32	32	≤8	>32	>32
9.	Cephalothin	>16	>16	≤8	≤8	>16	>16
10.	Chloramphenicol	≤8	≤8	≤8	≤8	>16	>16
11.	Ciprofloxacin	>2	>2	>2	>2	>2	>2
12.	Clindamycin	>2	>2	>2	>2	>2	>2
13.	Erythromycin	>4	>4	>4	≤0.5	>4	>4
14.	Gatifloxacin	4	4	≤2	≤2	>4	>4
15.	Gentamicin	>8	>8	>8	>8	>8	>8
16.	Imipenem	>8	>8	>8	≤4	>8	>8
17.	Levofloxacin	>4	>4	4	≤2	>4	>4
18.	Linezolid	≤2	≤2	≤2	≤2	>4	≤2
19.	Moxifloxacin	≤2	≤2	≤2	≤2	>4	>4
20.	Nitrofurantoin	≤32	≤32	≤32	≤32	≤32	≤32
21.	Norfloxacin	>8	>8	>8	>8	>8	>8
22.	Ofloxacin	>4	>4	4	4	>4	>4
23.	Oxacillin	>2	>2	>2	>2	>2	>2
24.	Penicillin	>8	>8	8	8	>8	>8
25.	Rifampin	>2	>2	>2	>2	>2	>2
26.	Synercid	≤1	≤1	≤1	≤1	≤1	≤1
27.	Tetracycline	>8	>8	≤4	≤4	>8	>8
28.	Trimethoprim/sulfamethoxazole	>2/38	>2/38	>2/38	>2/38	>2/38	>2/38
29.	Vancomycin	≤2	≤2	≤2	≤2	4	≤2

MIC values are presented in µg/mL; C: Control; T: Treatment; LS: Lab Isolate; LS 18: *S. haemolyticus*; LS 21: *S. epidermidis*; LS 30: *S. aureus*.

3.2. Identification by Biochemical and Biotype Number Study

Biochemical study results of control and biofield treated strains of *Staphylococcus* species are summarized in Table 3 and Table 4. Results showed that overall 25% change in tested biochemical reactions among three tested MDR strains of *Staphylococcus* species as compared to respective control. Rambrose changed from (+) positive to (-) negative reaction with respect to control in *S. haemolyticus* (LS 18). However, biochemicals such as acidification lactose, glycosidase, urea, and Voges-Proskauer changed from (-) negative to (+) positive reaction in LS 18 as compared to control. Only one biochemical *i.e.* Voges-Proskauer showed (-) negative to (+) positive reaction in *S. epidermidis* (LS 21) as compared to control. Basic characteristics of *S. aureus* are colony pigment, free coagulase, clumping factor, protein A, heat-stable nuclease, and acid production from mannitol. Crystal violet is the characteristic positive feature of *S. aureus*, but after biofield treatment in *S. aureus* (LS 30), it was changed from (+) positive to (-) negative reaction. Novobiocin changed from (+) positive to (-) negative reaction in LS 30 with respect to control. Rest of the biochemical reactions did not show any alteration after biofield treatment.

Biochemical reactions of control MDR strains of *Staphylococcus* species were well supported with literature

data [28, 29]. Based on the biochemical results, significant alteration in biotype number was observed in all three strains *i.e.* LS 18, LS 21, and LS 30 as compared to respective control. Biofield treatment in LS 18 showed an alteration in biotype number (306366) with the identification of new organism as *Staphylococcus simulans* on day 10 with respect to control (302302) (Table 4). Control group of *S. epidermidis* (LS 21) and *S. aureus* (LS 30) strains showed biotype number as 303064 and 757153 respectively. After biofield treatment LS 21 and LS 30 showed altered biotype number as 307064 and 317153 respectively (Table 4). Biofield treatment was recently used on pathogenic microbes to alter the biotype number on the basis of changed biochemical reactions [11, 21].

Biofield energy treatment as an alternate and complementary medicine, now included in subcategory of energy therapies by National Center for Complementary and Alternative Medicine/National Institute of Health (NCCAM/NIH) [30]. Mr. Trivedi's biofield treatment on pathogenic microbes was extensively studied and observed for alteration in the antimicrobial sensitivity pattern, biochemical reactions, and biotype number [10, 11]. Biofield treatment might involve alteration at genetic level and/or enzymatic level, which could hamper the drug ligand-receptor/protein interactions. Hence, a cascade of intracellular signals may be initiated, accelerated or inhibited

[31]. The overall study data showed that, biofield treatment on MDR strains of *Staphylococcus* species improved the sensitivity pattern and decrease in MIC value of 20.69%

tested antimicrobial, alter biochemical reactions, and biotype number.

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

S. No.	Code	Biochemical	LS 18		LS 21		LS 30	
			C	T	C	T	C	T
1.	ARA	Arabinose	-	-	-	-	-	-
2.	ARG	Arginine	+	+	-	-	+	+
3.	BAC	Bacillosamine	+	+	+	+	+	+
4.	BE	Bile esculin	-	-	-	-	-	-
5.	BL	β -lactamases	+	+	+	+	+	+
6.	CV	Crystal violet	-	-	-	-	+	-
7.	IDX	Indoxyl phosphatase	-	-	-	-	+	+
8.	INU	Inulin	-	-	-	-	-	-
9.	LAC	Acidification lactose	-	+	+	+	-	-
10.	MAN	Mannitol	-	-	-	-	+	+
11.	MNS	Mannose	-	-	-	-	+	+
12.	MS	Micrococcus screen	+	+	+	+	+	+
13.	NACL	Sodium chloride	+	+	+	+	+	+
14.	NIT	Nitrate	+	+	+	+	+	+
15.	NOV	Novobiocin	-	-	-	-	+	-
16.	OPT	Optochin	+	+	+	+	+	+
17.	PGR	Glycosidase*	-	-	-	-	-	-
18.	PGT	Glycosidase#	-	+	+	+	+	+
19.	PHO	Phosphatase	-	-	+	+	+	+
20.	PRV	Pyruvate	+	-	-	-	-	-
21.	PYR	Pyrolidonyl arylamidase	+	+	-	-	-	-
22.	RAF	Raffinose	-	-	-	-	-	-
23.	RBS	Rambose	+	-	-	-	-	-
24.	SOR	Sorbitol	-	-	-	-	-	-
25.	TFG	Thymidine free growth	+	+	+	+	+	+
26.	TRE	Acidification trehalose	+	+	-	-	+	+
27.	URE	Urea	-	+	+	+	-	-
28.	VP	Voges-Proskauer	-	+	-	+	+	+

C: Control; T: Treatment; LS: Lab Isolate; LS 18: *S. haemolyticus*; LS 21: *S. epidermidis*; LS 30: *S. aureus*; -: negative; +: positive; *PGR: p-nitro phenyl β -D-glucuronide; #PGT: p-nitro phenyl β -D-galactopyranoside.

Table 4. Effect of biofield treatment on multidrug resistant lab isolates of *Staphylococcus* spp. to distinguishing feature of the genotype.

Isolate	Group	Biotype Number	Organism Identification
LS 18	C	302302	<i>Staphylococcus haemolyticus</i>
	T	306366	<i>Staphylococcus simulans</i>
LS 21	C	303064	<i>Staphylococcus epidermidis</i>
	T	307064	<i>Staphylococcus epidermidis</i>
LS 30	C	757153	<i>Staphylococcus aureus</i>
	T	317153	<i>Staphylococcus aureus</i>

C: Control; T: Treatment; LS: Lab Isolate; LS 18: *S. haemolyticus*; LS 21: *S. epidermidis*; LS 30: *S. aureus*

4. Conclusions

Overall results suggested the impact of biofield treatment on *Staphylococcus* species. Antimicrobial sensitivity results in improved sensitivity pattern of erythromycin, with eight-fold decrease in MIC value (*i.e.* >4 to ≤ 0.5 $\mu\text{g/mL}$), while, levofloxacin was also reported with improved sensitivity pattern with improved MIC value (4 to ≤ 2 $\mu\text{g/mL}$) in biofield treated *S. epidermidis*. Biofield treatment on *Staphylococcus* species showed altered effect on 25% tested biochemicals and biotype numbers. A significant change in biotype number (306366) on the basis of altered biochemical reactions as

compared to control (302302). Altered biotype number results in identification of new organism as *Staphylococcus simulans* with respect to control species as *S. haemolyticus* in LS 18 after biofield treatment. Based on the results, Mr. Trivedi's biofield energy treatment could be applied to improve the sensitivity pattern of antimicrobials, against multidrug resistance strains of *Staphylococcus* species.

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