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Antibiogram, Biochemical Reactions and Genotyping Characterization of Biofield Treated *Staphylococcus aureus*

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Abstract: *Staphylococcus aureus* (*S. aureus*) is the key organism for food poisoning due to massive production of heat stable exotoxins. The current study was attempted to investigate the effect of Mr. Trivedi's biofield treatment on *S. aureus*. *S. aureus* (ATCC 25923) was divided into two parts, Group (Gr.) I: control and Gr. II: treatment. After biofield treatment, Gr. II was further subdivided into two parts, Gr. IIA and Gr. IIB. Gr. IIA was analyzed on day 10, while Gr. IIB was stored and analyzed on day 159 after revival (Study I). The revived sample (Gr. IIB) were retreated on day 159 (Study II), and divided into three separate tubes. Tube 1 was analyzed on day 5, likewise, tube 2 and 3 were analyzed on day 10 and 15, respectively. All the experimental parameters were studied using automated MicroScan Walk-Away® system. The 16S rDNA sequencing was carried out in Gr. IIA sample to correlate the phylogenetic relationship of *S. aureus* with other bacterial species. The antimicrobial susceptibility and minimum inhibitory concentration showed significant alteration *i.e.* 92.86% and 90.00% respectively in treated cells of *S. aureus* as compared to control. The biochemical reactions also showed the significant (35.71%) alteration in treated sample with respect to control. The biotype number and microbial species were substantially changed in Gr. IIA (767177; *Staphylococcus cohnii* subsp. *urealyticum*) on day 10, while only the biotype numbers were changed in rest of the treated samples as compared to control (307016; *S. aureus*). The 16S rDNA analysis showed that the identified strain in this experiment was *S. aureus* (GenBank Accession No.: L37597) after biofield treatment. However, the nearest homolog genus-species was found as *Staphylococcus simiae* (GenBank Accession No.: DQ127902). These results suggested that biofield treatment has a significant impact on *S. aureus* in lyophilized as well as revived state.

Keywords: Staphylococci, *Staphylococcus aureus*, Antimicrobial Sensitivity, Biofield Treatment, Biochemical Reaction, Biotype, 16S rDNA, Gram-Positive Bacteria

1. Introduction

Staphylococci are the important class of pyogenic Gram-positive spherical bacteria resembling to the grapes like structure. They are considered as the third most important cause of food-borne disorders in the world [1]. It is the main pathogen for mastitis in the milch animals [2]. It is estimated that in US alone food-borne illnesses affect 6 to 80 million people each year, causing up to 9000 deaths [3]. Based on literature various genes have been found as a target for identification of *S. aureus* with the help of 16S rDNA sequence *viz.* heat shock protein 60 (*hsp60*) [4], superoxide dismutase A (*sodA*) [5], and RNA polymerase B (*rpoB*) [6]. *S. aureus* has developed resistance to the most classes of the antimicrobial agents. Penicillin is the drug of choice to treat against *Staphylococcus* infection but due to penicillinase or β-lactamase enzyme that destroy the penicillin, leads to resistance against *S. aureus* [7]. Therefore, some alternative strategies are needed to treat against staphylococci infections.

National Institute of Health/National Center for Complementary and Alternative Medicine (NIH/NCCAM) have reported that biofield (putative energy fields) or electromagnetic based energy therapies used to promote

health and healing [8]. Biofield energy treatment has been known as an alternative approach that may be useful to alter the sensitivity pattern of the antimicrobials. Harold Saxton Burr had performed the detailed studies on the correlation of electric current with physiological processes and suggested that every single process in the human body had an electrical significance [9]. The electrical process that happening in the human body have strong relationship with magnetic field as required by Ampere's law, which stated that the moving charge produces magnetic field in surrounding space [10, 11]. Thus, the human body emits the electromagnetic waves in the form of bio-photons, which surrounds the body and it is commonly known as biofield. Therefore, the biofield consists of an electromagnetic field, being generated by moving electrically charged particles (ions, cell, molecule, etc.) inside the human body. Prakash *et al.* 2015, reported that the various scientific instruments such as Kirlian photography, polycontrast interference photography and resonance field imaging can be extensively used to measure the biofield of human body [12]. Thus, a 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 respond into useful way that is called biofield energy and the process is known as biofield treatment. Mr. Trivedi's biofield treatment (The Trivedi Effect®) has been known to alter the structural, physical and thermal properties of several metals in materials science [13-15], improved the overall productivity of crops [16, 17], altered characteristics features of microbes [18-20] and improved growth and anatomical characteristics of various medicinal plants [21, 22]. 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 modality on *S. aureus* in relation to the antimicrobials susceptibility, biochemical reactions, biotyping and 16S rDNA sequencing.

2. Materials and Methods

S. aureus, American Type Culture Collection (ATCC 25923) 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 Positive Breakpoint Combo 20 (PBPC 20) panel. The 16S rDNA sequencing analysis was carried out using ultrapure genomic DNA prep kit; Cat KT 83 (Bangalore Genei, India).

2.1. Experimental Design

The impact of biofield treatment on tested bacterium *S. aureus* was evaluated in two groups-

Group I: ATCC strain in lyophilized state was considered as control. No treatment was given and analyzed for antimicrobial sensitivity, biochemical reactions and biotype number as per the standard protocol.

Group II: The lyophilized state of ATCC strain was divided into two parts named as Gr. IIA and Gr. IIB. Both the groups of ATCC strain of *S. aureus* in lyophilized state were assigned to the Mr. Trivedi's unique biofield treatment (first treatment). Gr. IIA was analyzed on day 10 while Gr. IIB sample was stored in lyophilized state for 159 days at -70ºC. Gr. IIB was further sub-divided in two separate parts named as Gr. IIB - Study I and Gr. IIB - Study II.

Group IIB - Study I

After 159 days, antimicrobial sensitivity, MIC, biochemical reactions and biotyping were performed as per the standard protocol.

Group IIB - Study II

The stored strain was revived from -70ºC and the revived culture was again provided to Mr. Trivedi's biofield treatment (re-treatment) on day 159. After biofield retreatment, the sample was sub-cultured into three separate tubes and analyzed on Day 5, Day 10 and Day 15 of its sub-culturing.

2.2. Biofield Treatment Strategy

The lyophilized sample of *S. aureus* was subjected to Mr. Trivedi's biofield treatment (first treatment) and then stored, analyzed on day 10 (Gr. IIA) followed by retreatment on 159 days in revived state (Gr. IIB, Study II). In details, the treatment groups 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 group without touching the samples. After first treatment, the analysis of Gr. IIA lyophilized sample was done on day 10 for antimicrobial sensitivity along with minimum inhibitory concentration (MIC), biochemical reactions with biotype number and 16S rDNA analysis as per the standard protocol. While handing over these cultures to Mr. Trivedi for retreatment purposes, optimum precautions were taken to avoid contamination.

2.3. Antimicrobial Susceptibility Test

Investigation of antimicrobial susceptibility of *S. aureus* was carried out with the help of automated instrument, MicroScan Walk-Away® using PBPC 20 panel. The panel can be stored at 2 to 25ºC for analysis. The panel was allowed to equilibrate to room temperature prior to rehydration. All opened panels were used on the same day. The tests carried out on MicroScan were miniaturized of the broth dilution susceptibility test that has been dehydrated. Briefly, 0.1 mL of the standardized suspension of *S. aureus* 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 were performed using the

RENOK® system with inoculators-D (B1013-4). 25 mL of standardized inoculum suspension was poured into inoculum tray. The detailed experimental procedure and conditions were followed as per the manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, R: Resistant; and BLAC: β-lactamase positive) and MIC were determined by observing the lowest antimicrobial concentration showing inhibition of growth [23].

2.4. Biochemical Reaction Studies

Biochemical reactions of *S. aureus* were determined using MicroScan Walk-Away®, system with PBPC 20 panel. Preparation of PBPC 20 panel, inoculum followed by dehydration and rehydration were performed in a similar way as mentioned in antimicrobial susceptibility assay for analysis of biochemical reactions followed by biotype number. The detailed experimental procedures and conditions were followed as per the manufacturer's instructions [23].

2.5. Identification of Organism by Biotype Number

The biotype number of *S. aureus* was determined on MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data [23, 24].

2.6. Amplification and Gene Sequencing of 16S rDNA

Genomic DNA was isolated from *S. aureus* cells (Gr. IIA, sample coded as 9A) using genomic purification kit, according to the manufacturer instructions. 16S rDNA gene (~1.5 kb) fragment was amplified with the help of high-fidelity polymerase chain reaction (PCR) using universal primers; forward primer (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer (3′-ACGGTCATACCTTGTTACGACTT-5′). Amplified products were subjected to gel electrophoresis in 1.0% agarose gel, stained with ethidium bromide and visualized under UV light in a gel documentation unit (BioRad Laboratories, USA). The PCR amplified fragment was purified from the agarose gel using a DNA gel extraction kit. Sequencing of amplified product was done on a commercial basis from Bangalore Genei, India. The 16S rDNA sequences obtained were aligned and compared with the sequences stored in GenBank database available from National Center for Biotechnology Information (NCBI) using the algorithm BLASTn program. Multiple sequence alignment / phylogenetic tree were established using MEGA3.1 molecular software [25].

3. Results and Discussion

3.1. Antimicrobial Susceptibility Test

The results of *S. aureus* susceptibility pattern and MIC values of tested antimicrobials after biofield treatment are summarized in Table 1 and 2 respectively. The data were analyzed and compared with respect to control. Antimicrobial sensitivity assay and MIC were performed in twenty-eight and thirty antimicrobials respectively. The treated cells of *S. aureus* showed a significant (85.71%) alteration (twenty-four out of twenty-eight) in antimicrobial sensitivity pattern from susceptible (S) to resistance (R) in lyophilized treated Gr. IIA on day 10 after first-time biofield treatment as compared with control. However, these twenty-four antimicrobials did not show any change of sensitivity pattern on day 159 as well as in revived state even after retreatment as compared to control. Out of twenty-eight antimicrobials two antibiotics *i.e.* ampicillin and penicillin were changed from S to β-lactamase positive (BLAC) in lyophilized treated Gr. IIA on day 10 while showed similar response in rest of treated groups even after second-time biofield treatment as compared with control. *S. aureus* has the ability to produce β-lactamases or penicillinase enzyme which breakdown the β-lactam ring present in penems and cephems heteronucleus [26]. Two, out of twenty eight (7.14%) tested antimicrobials such as piperacillin/tazobactam and linezolid did not show any responses in lyophilized treated cells (Gr. IIA) on day 10 while they exhibited susceptible in rest of treated samples of *S. aureus*. Overall, 92.86% antimicrobial susceptibility pattern was altered after biofield treatment as compared to control. MIC values of several antimicrobials *viz.* ampicillin/sulbactam, azithromycin, cefazolin, cefepime, cephalothin, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, levofloxacin, linezolid, moxifloxacin, norfloxacin, ofloxacillin, rifampin, tetracycline, and synercid showed an alteration about two-fold in Gr. IIA on day 10 as compared to control. The MIC value of cefotaxime, ceftriaxone and clindamycin were changed about four-fold in Gr. IIA on day 10 while remained unchanged in rest of the groups as compared to control.

Certain antimicrobials such as erythromycin, oxacillin and vancomycin showed eight-fold, while ampicillin showed thirty two-fold (≤0.25 to >8 µg/mL) and penicillin showed around two hundred sixty seven-fold (≤0.03 to >8 µg/mL) alteration of MIC values in Gr. IIA on day 10 as compared to control. Amoxicillin / k-clavulanate and trimethoprim / sulfamethoxazole were slightly altered the MIC values in Gr. IIA on day 10. Overall, 90% out of thirty tested antimicrobials showed an alteration of MIC values as compared to control. Three out of thirty (10%) antimicrobials did not show any alteration of MIC values in all the treated groups as compared to control (Table 2) except piperacillin / tazobactam in Gr. IIA, value not reported. Overall, the antimicrobial resistance pattern (S to R) and corresponding MIC values were significantly altered in lyophilized strain *S. aureus* after first-time biofield treatment as compared to control.

3.2. Biochemical Reactions Studies

Data obtained from biochemical reactions studies for distinction of *S. aureus* are illustrated in Table 3. Study of biochemical reactions can be utilized to identify the enzymatic and metabolic characteristic feature of microbes.

Microorganisms can be categorically differentiated based on their utilization of specific biochemicals as nutrients during the process of metabolism or enzymatic reactions. Biochemicals such as arginine (ARG), mannose (MNS) and urea (URE) were changed from negative (-) to positive (+) reactions in all the treated groups with respect to control. Moreover, biochemical reactions of β-lactamases (BL), crystal violet (CV), novobiocin (NOV), galactosidase (PGR and PGT) and sorbitol (SOR) were converted from negative (-) to positive (+) reactions in Gr. IIA on day 10 after first biofield treatment and remained same *i.e.* negative (-) in rest of the treated samples as compared to control. The alterations of biochemical reactions after biofield treatment of ARG, PGR and PGT were the typical characteristics feature of *S. aureus*. Based on this data, it is assumed that Mr. Trivedi's biofield treatment has an impact on *S. aureus* in term of metabolic reaction. Similarly, rambose (RBS) was converted from negative (-) to the positive (+) reaction after first-time biofield treatment in Gr. IIA on day 10 and after retreatment in Gr. IIB, Study II on day 15 while remained unchanged in rest of the treated groups as compared to control. The key characteristic feature for *S. aureus* are colony pigment, free coagulase, clumping factor, protein A, heat-stable nuclease and acid production from mannitol [27]. In this experiment, due to the production of acid from mannitol, the result showed positive (+) reaction in all the treated groups which supports the metabolic characteristics feature of S. *aureus*. Overall, 35.71% biochemical reactions were altered in tested twenty-eight biochemicals with respect to control after biofield treatment. About 64.29% of total biochemicals, such as arabinose, bacillosamine, bile esculin, indoxyl phosphatase, inulin, acidification lactose, mannitol, micrococcus screen, sodium chloride, nitrate, optochin, phosphatase, pyruvate, pyrolidonyl arylamidase, raffinose, thymidine free growth, acidification trehalose and Voges-Proskauer did not show any change in all the groups after biofield treatment as compared to control.

Table 1. *Antibiogram of Staphylococcus aureus: effect of biofield treatment on antimicrobial susceptibility.*

S. No.	Antimicrobial	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
					Day 5	Day 10	Day 15
1.	Amoxicillin/k-clavulanate	S	R	S	S	S	S
2.	Ampicillin/sulbactam	S	R	S	S	S	S
3.	Ampicillin	S	BLAC	S	S	S	S
4.	Azithromycin	S	R	S	S	S	S
5.	Cefazolin	S	R	S	S	S	S
6.	Cefepime	S	R	S	S	S	S
7.	Cefotaxime	S	R	S	S	S	S
8.	Ceftriaxone	S	R	S	S	S	S
9.	Cephalothin	S	R	S	S	S	S
10.	Chloramphenicol	S	R	S	S	S	S
11.	Ciprofloxacin	S	R	S	S	S	S
12.	Clindamycin	S	R	S	S	S	S
13.	Erythromycin	S	R	S	S	S	S
14.	Gatifloxacin	S	R	S	S	S	S
15.	Gentamicin	S	R	S	S	S	S
16.	Imipenem	S	R	S	S	S	S
17.	Levofloxacin	S	R	S	S	S	S
18.	Linezolid	S	NA	S	S	S	S
19.	Moxifloxacin	S	R	S	S	S	S
20.	Ofloxacillin	S	R	S	S	S	S
21.	Oxacillin	S	R	S	S	S	S
22.	Penicillin	S	BLAC	S	S	S	S
23.	Piperacillin/tazobactam	S	–	S	S	S	S
24.	Rifampin	S	R	S	S	S	S
25.	Synercid	S	R	S	S	S	S
26.	Tetracycline	S	R	S	S	S	S
27.	Trimethoprim/sulfamethoxazole	S	R	S	S	S	S
28.	Vancomycin	S	R	S	S	S	S

R: Resistant; S: Susceptible; Gr.: Group; BLAC: β-lactamase positive; NA: Data not available;
-, Not reported

Table 2. *Effect of biofield treatment on Staphylococcus aureus to minimum inhibitory concentration (MIC) of tested antimicrobials.*

S. No.	Antimicrobial	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
					Day 5	Day 10	Day 15
1.	Amoxicillin/k-clavulanate	≤4/2	>4/2	≤4/2	≤4/2	≤4/2	≤4/2
2.	Ampicillin/sulbactam	≤8/4	>16/8	≤8/4	≤8/4	≤8/4	≤8/4
3.	Ampicillin	≤0.25	>8	≤0.25	≤0.25	≤0.25	≤0.25
4.	Azithromycin	≤2	>4	≤2	≤2	≤2	≤2
5.	Cefazolin	≤8	>16	≤8	≤8	≤8	≤8
6.	Cefepime	≤8	>16	≤8	≤8	≤8	≤8
7.	Cefotaxime	≤8	>32	≤8	≤8	≤8	≤8
8.	Ceftriaxone	≤8	>32	≤8	≤8	≤8	≤8
9.	Cephalothin	≤8	>16	≤8	≤8	≤8	≤8
10.	Chloramphenicol	≤8	>16	≤8	≤8	≤8	≤8
11.	Ciprofloxacin	≤1	>2	≤1	≤1	≤1	≤1
12.	Clindamycin	≤0.5	>2	≤0.5	≤0.5	≤0.5	≤0.5
13.	Erythromycin	≤0.5	>4	≤0.5	≤0.5	≤0.5	≤0.5
14.	Gatifloxacin	≤2	>4	≤2	≤2	≤2	≤2
15.	Gentamicin	≤4	>8	≤4	≤4	≤4	≤4
16.	Imipenem	≤4	≤4	≤4	≤4	≤4	≤4
17.	Levofloxacin	≤2	>4	≤2	≤2	≤2	≤2
18.	Linezolid	≤2	>4	≤2	≤2	≤2	≤2
19.	Moxifloxacin	≤2	>4	≤2	≤2	≤2	≤2
20.	Nitrofurantoin	≤32	≤32	≤32	≤32	≤32	≤32
21.	Norfloxacin	≤4	>8	≤4	≤4	≤4	≤4
22.	Ofloxacillin	≤2	>4	≤2	≤2	≤2	≤2
23.	Oxacillin	≤0.25	>2	≤0.25	≤0.25	≤0.25	≤0.25
24.	Penicillin	≤0.03	>8	≤0.03	≤0.03	≤0.03	≤0.03
25.	Piperacillin/tazobactam	≤4	–	≤4	≤4	≤4	≤4
26.	Rifampin	≤1	>2	≤1	≤1	≤1	≤1
27.	Synercid	≤1	>2	≤1	≤1	≤1	≤1
28.	Tetracycline	≤4	>8	≤4	≤4	≤4	≤4
29.	Trimethoprim/sulfamethoxazole	≤2/38	>2/38	≤2/38	≤2/38	≤2/38	≤2/38
30.	Vancomycin	≤2	>16	≤2	≤2	≤2	≤2

MIC data are presented in µg/mL; Gr.: Group; -, Not reported

Table 3. *Effect of biofield treatment on Staphylococcus aureus to the biochemical reaction pattern.*

S. No.	Code	Biochemical	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
						Day 5	Day 10	Day 15
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	+	+	+	+	+	+

-, (negative); +, (positive); Gr.: Group; *PGR: p-nitro phenyl β-D- glucuronide; #PGT: p-nitro phenyl β-D-galactopyranoside

3.3. Identification of Organism by Biotype Number

The species (*S. aureus*) 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 an automated system, and results showed a significant change in biotype number (767177) in Gr. IIA (on day 10) after first-time biofield treatment with identification of new species (*Staphylococcus cohnii* subsp. *urealyticum*) as compared to control Gr. I (307016; *S. aureus*). Based on the biochemical results, biotype numbers were also changed in rest of treated groups without alteration of species with respect to control (307016) *i.e. S. aureus* (Table 4).

Table 4. *Effect of biofield treatment on biotype number of Staphylococcus aureus.*

Feature	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 159)	Gr. IIB (Study II; Day 159)		
				Day 5	Day 10	Day 15
Biotype	307016	767177	307137	307137	307137	307137
Organism Identification	*S. aureus*	*Staphylococcus cohnii* subsp. *urealyticum*	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*

Gr.: Group

3.4. 16S rDNA Genotyping

The bacteria that are poorly differentiated by conventional methods needs molecular analysis method like 16S rDNA sequence [28]. This molecular-based technique is a suitable tool for identification of most of the bacteria on their genus and/or species level by comparison with databases in the public domain. Because, most of the bacteria possess small ribosomal subunit with species-specific variability [29]. The 16S rDNA analysis was performed after first-time biofield treated sample (Gr. IIA) of *S. aureus* on day 10. The alignment and comparison of the consensus gene sequences were performed with the sequences stored in GenBank database available from NCBI using the algorithm BLASTn program. Based on nucleotide homology and phylogenetic analysis the microbe (Sample 9A) was detected as *Staphylococcus aureus* (GenBank Accession No: L37597) with 100% identity. The nearest homolog genus-species of *S. aureus* was found as *Staphylococcus simiae* (GenBank Accession No. DQ127902). Some other close homologs of *S. aureus* were found from the alignment results as shown in Table 5. The distance matrix based on nucleotide sequence homology data are presented in Table 6. The phylogenetic tree was established using BLAST-Webpage (NCBI). According to Table 6, ten different related bacterial species of *S. aureus* were selected as Operational Taxonomic Units (OTUs) in order to investigate the phylogenetic relationship of *S. aureus*. There were 1497 base nucleotides of 16S rDNA gene sequences, which were analyzed and multiple alignments were constructed using ClustalW in MEGA3.1. The numbers of base substitutions per site from pairwise distance analysis between sequences are shown in Table 5.

All results were based on the pairwise analysis of 11 sequences. According to the data in Table 6, the lowest value of the genetic distance from *S. aureus* was 0.000 base substitutions per site. This value is due to the distance between *S. aureus* and *Staphylococcus simiae*. All pairwise distance analysis was carried out using the p-distance method in MEGA3.1. The proportion of remarked distance, sometimes also called p-distance and showed as the number of nucleotide distances site. Values in Table 5 are programmed into Figure 1 with optimal bootstrap consensus tree. In the phylogram, there were eleven OTUs. The results suggested that *S. aureus* was closely related to the *Staphylococcus simiae* with 100% similarity and the lowest genetic distance 0.000 base substitutions per site.

Table 5. *The closest sequences of Staphylococcus aureus from sequence alignment using NCBI GenBank and ribosomal database project (RDP).*

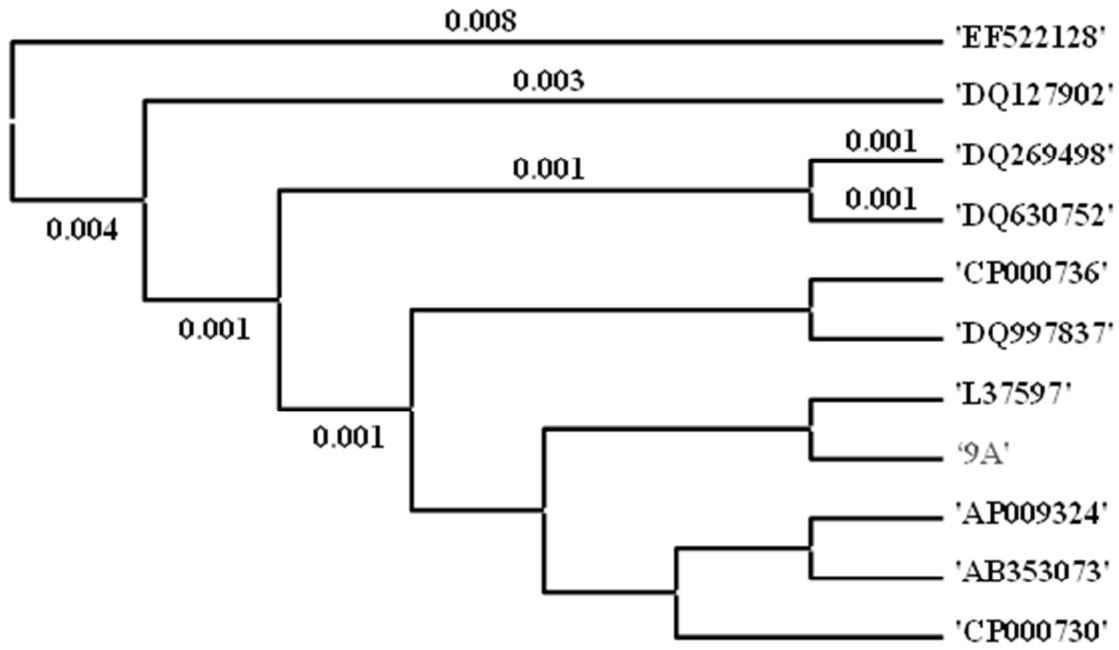
Alignment View	AN	Alignment Result	Sequence Description
	9A	1.00	Sample studied
	CP000730	1.00	*Staphylococcus aureus* subsp. *aureus* USA300
	AP009324	1.00	*Staphylococcus aureus* subsp. *aureus* Mu3
	CP000736	1.00	*Staphylococcus aureus* subsp. *aureus* JH1
	AB353073	1.00	*Staphylococcus aureus* strain: MPU99
	L37597	1.00	*Staphylococcus aureus*
	DQ997837	1.00	*Staphylococcus aureus* strain ATCC 14458
	DQ269498	0.99	*Staphylococcus aureus* strain ATCC 14458
	DQ630752	0.99	*Staphylococcus aureus* isolation-source bhalla
	DQ127902	0.97	*Staphylococcus simiae* strain CCM 7229
	EF522128	0.99	*Staphylococcus epidermidis* strain CU22

AN: GenBank Accession Number

Table 6. *Distance matrix Staphylococcus aureus sample (9A) based on nucleotide sequence homology (Using Kimura-2 Parameter) indicates nucleotide similarity (above diagonal) and distance (below diagonal) identities between the studied sample '9A' and ten closest homologs microbe.*

Distance Matrix												
AN		1	2	3	4	5	6	7	8	9	10	11
CP000736	1	—	1	1	0.998	1	0.998	1	0.995	0.986	1	1
DQ997837	2	0.000	—	1	0.998	1	0.998	1	0.995	0.986	1	1
AP009324	3	0.000	0.000	—	0.998	1	0.998	1	0.995	0.986	1	1
DQ269498	4	0.002	0.002	0.002	—	0.998	0.999	0.998	0.995	0.984	0.998	0.998
AB353073	5	0.000	0.000	0.000	0.002	—	0.998	1	0.995	0.986	1	1
DQ630752	6	0.002	0.002	0.002	0.001	0.002	—	0.998	0.995	0.984	0.998	0.998
CP000730	7	0.000	0.000	0.000	0.002	0.000	0.002	—	0.995	0.986	1	1
DQ127902	8	0.005	0.005	0.005	0.005	0.005	0.005	0.005	—	0.985	0.995	0.995
EF522128	9	0.014	0.014	0.014	0.016	0.014	0.016	0.014	0.015	—	0.986	0.986
L37597	10	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.005	0.014	—	1
9A	11	0.000	0.000	0.000	0.002	0.000	0.002	0.000	0.005	0.014	0.000	—

AN: GenBank Accession Number



Numbers represent GenBank accession number

Figure 1. *Phylogenetic tree of the partial 16S rDNA gene sequencing of Staphylococcus aureus using MEGA 3.1 software using neighbor joining method. Numbers represent GenBank accession number.*

Biofield treatment may 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 [30]. Biofield treatment might induce significant changes in lyophilized strain of *S. aureus* 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 BLAC/resistant in treated cells of *S. aureus* predominately after biofield treatment. Due to microbial resistance to a single drug or multiple drugs, the invention of an effective antimicrobial therapy for the human-wellness is urgently required. However, due to some limitation of science, the progress of new medications is slow and very challenging for scientists. So far our group had published many research articles regrading short-term effects on biofield treatment on ATCC and multidrug resistant strains [18-20]. Based on these results, it is envisaged that biofield treatment has the ability to alter the sensitivity pattern of antimicrobials and scope to be an alternative approach than the existing antimicrobial therapy in near future.

4. Conclusions

In conclusion, the antimicrobial susceptibility pattern and the MIC values showed the significant 92.86% and 90% alteration, respectively of tested antimicrobials as compared

to the control strain of *S. aureus*. The biochemical reactions pattern showed the significant 35.71% alteration as compared to the control. Moreover, the biotype numbers of biofield treated strain of *S. aureus* were also changed in all the treated groups as compared to the control. Based on the changed biotype numbers after biofield treatment, new species was identified as (767177; *Staphylococcus cohnii* subsp. *urealyticum*) in lyophilized treated cells (Gr. IIA) on day 10 with respect to the control Gr. I (307016; *S. aureus*). Thus, Mr. Trivedi's unique biofield energy treatment could be applied as an alternative therapeutic approach against antimicrobials to alter the sensitivity pattern. Molecular based 16S rDNA analysis showed that the treated lyophilized sample in this experiment was *S. aureus*. However, the nearest homolog genus-species was found to be *Staphylococcus simiae*. Based on these results, it seems that biofield treatment could be used as an alternate of existing drug therapy in future.

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