

Antimicrobial Sensitivity, Biochemical Characteristics and Biotyping of *Staphylococcus saprophyticus*: An Impact of Biofield Energy Treatment

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Abstract

Staphylococcus saprophyticus (*S. saprophyticus*) is a frequent cause of urinary tract infection in the young women. The current study was designed to analyze the effect of biofield energy treatment on *S. saprophyticus* for evaluation of its antibiogram profile, biochemical reactions pattern and biotyping characteristics. Two sets of ATCC samples were taken in this experiment and denoted as A and B. Sample A was revived and divided into two parts Group (Gr.I) (control) and Gr.II (revived); likewise, sample B was labeled as Gr.III (lyophilized). Gr. II and III were given with Mr. Trivedi's biofield energy treatment. The control and treated groups of *S. saprophyticus* cells were tested with respect to antimicrobial susceptibility, biochemical reactions pattern and biotype number using MicroScan Walk-Away[®] system. The 50% out of twenty-eight tested antimicrobials showed significant alteration in susceptibility and 36.67% out of thirty antimicrobials showed an alteration in minimum inhibitory concentration (MIC) value of *S. saprophyticus* in revived treated cells (Gr. II, day 10), while no alteration was found in lyophilized treated cells (Gr. III, day 10) as compared to the control. It was also observed that overall 14.81%, out of twenty-seven biochemical reactions were altered in the revived treated group with respect to the control. Moreover, biotype number was changed in Gr. II, on day 5 (246076) and in Gr. III, on day 10 (242066), while organism along-with biotype number was also changed in Gr. II, on day 10 (342066, *Staphylococcus hominis* subsp. *novobiosepticus*) as compared to the control (242076, *S. saprophyticus*). The result suggested that biofield treatment has the significant impact on *S. saprophyticus* in revived treated cells with respect to the antimicrobial susceptibility, MIC, biochemical reactions pattern and biotype.

Keywords: *Staphylococcus saprophyticus*; Antimicrobial susceptibility; Biofield energy treatment; Biochemical reaction; Biotype; Antibiogram; Gram-positive

Abbreviations: NIH/NCCAM: National Institute of Health/National Center for Complementary and Alternative Medicine; ATCC: American Type Culture Collection; PBPC 20: Positive Breakpoint Combo 20; MIC: Minimum Inhibitory Concentration; CoNS: Coagulase-negative staphylococci; UTIs: Urinary tract infections

Introduction

Staphylococcus saprophyticus (*S. saprophyticus*) is a Gram-positive, coagulase-negative facultative bacterium belongs to *Micrococcaceae* family. It is a unique uropathogen associated with uncomplicated urinary tract infections (UTIs), especially cystitis in young women. Young women are very susceptible to colonize this organism in the urinary tracts and it is spread through sexual intercourse. *S. saprophyticus* is the second most common pathogen after *Escherichia coli* causing 10-20% of all UTIs in sexually active young women [1-3]. It contains the urease enzymes that hydrolyze the urea to produce ammonia. The urease activity is the main factor for UTIs infection. Apart from urease activity it has numerous transporter systems to adjust against change in pH, osmolarity, and concentration of urea in human urine [2]. After severe infections, it causes various complications such as native valve endocarditis [4], pyelonephritis, septicemia, [5], and nephrolithiasis [6]. About 150 million people are diagnosed with UTIs each year worldwide [7]. Several virulence factors includes due to the adherence to urothelial cells by release of lipoteichoic acid is a surface-associated adhesion amphiphile [8], a hemagglutinin that binds to fibronectin and hemagglutinates sheep erythrocytes [9], a hemolysin; and production of extracellular slime are responsible for resistance properties of *S. saprophyticus* [1]. Based on literature, *S. saprophyticus* strains are susceptible to vancomycin, rifampin, gentamicin and amoxicillin-clavulanic, while resistance to other antimicrobials such as erythromycin, clindamycin, fluoroquinolones, chloramphenicol, trimethoprim/sulfamethoxazole, oxacillin, and

penicillin [10]. An alternative i.e., biofield energy based healing therapy is recently reported to alter the antimicrobial sensitivity pattern in different microorganisms [11]. 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) [12]. The human body naturally emits the waves in the form of bio-photons, which surrounds the body and it is commonly known as biofield. In the recent year, Prakash et al. reported that 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 [13]. Although, a human has the capability to harness the energy from the environment or universe and can transmit it into any object(s) around the Globe. The objects always receive the energy and responding in a useful way that is called biofield energy and the process is called as biofield treatment. Mr. Trivedi's unique biofield energy treatment ('The Trivedi Effect') has been known to alter the characteristics features of pathogenic microbes [14,15], an improved growth and productivity of plants [16,17] and also able to alter the thermophysical properties of metal and ceramic in materials science [18,19]. Due to the clinical importance of *S. saprophyticus* and literature reports on biofield, this work was undertaken to evaluate the impact of

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biofield treatment in relation to the antimicrobials susceptibility and biotyping based on various biochemical characteristics.

Materials and Methods

S. saprophyticus, American Type Culture Collection (ATCC 15305) strains were procured from MicroBioLogics, Inc., USA, in two sets A and B. The antimicrobials and biochemicals were used in this experiment procured from Sigma-Aldrich, MA, USA. The antimicrobial susceptibility, biochemical reaction pattern 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.

Experimental Design

Two ATCC 15305 samples A and B of *S. saprophyticus* 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

The control sample (Gr. I) was remained as untreated. The treated groups, Gr. II and III were handed over in sealed pack to Mr. Trivedi for biofield energy treatment under laboratory conditions. Mr. Trivedi provided the treatment through his energy transmission process to the treated groups (Gr. II and Gr. III) without touching the samples. After treatment, all treated samples were stored for analysis in the same condition. Gr.II was assessed at two time point *i.e.* on day 5 and 10 and Gr. III was assessed on day 10 after the biofield treatment, for antimicrobial susceptibility, biochemical reactions pattern and biotyping.

Antimicrobial Susceptibility Test

The antimicrobial susceptibility of *S. saprophyticus* was carried out with the help of automated instrument, MicroScan Walk-Away[®] using PBPC 20 panel. The panel was allowed to equilibrate to room temperature before 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. saprophyticus* cultured cells were taken 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. After that, rehydration and followed by inoculation were performed using the RENOK[®] system with inoculators-D (B1013-4). Approximately 25 mL of standardized inoculum suspension was poured into the inoculum tray. The detailed experimental procedure and conditions were maintained as per the manufacturer's instructions. The antibiogram profile like as susceptible, resistant, β -lactamase positive (BLAC) and minimum inhibitory concentration (MIC) were determined [20].

Biochemical Reaction Studies

The biochemical reactions of *S. saprophyticus* were determined using MicroScan Walk-Away[®] system with PBPC 20 panel. Preparation of PBPC 20 panel, inoculum and followed by dehydration and rehydration were performed in a similar way as mentioned in the antimicrobial susceptibility assay for the analysis of biochemical reactions followed by biotype number. The MicroScan Walk-Away[®] system contains photometric or fluorogenic reader. On the basis of nature of bacilli (*i.e.* Gram-positive), computerized reports were generated using conventional panel, which utilizes the photometric

reader. Before commencing the experiment, the PBPC 20 panel was first incubated and read on the MicroScan Walkaway system. After evaluating the experimental reading on the Walkaway system, the PBPC 20 panel was removed from the system and recorded on the Biomic system within 1 h. The instrument consists of a database associated with collective information, which was required to identify the microbes with respect to group, genera, or species of the family. Detailed experimental procedure was followed as per manufacturer-recommended instructions [20].

Identification of Organism By Biotype Number

The biotype number of *S. saprophyticus* was determined on MicroScan Walk-Away[®] processed panel data report with the help of biochemical reactions data. The similar experimental procedure was followed for identification of biotype number as described in biochemical reaction study, and as per manufacturer-recommended instructions [20].

Results and Discussion

Antimicrobial susceptibility test

The data obtained in this experiment related to antimicrobials sensitivity profile and MIC values are illustrated in Tables 1 and 2, respectively. After biofield energy treatment, the data were analyzed and compared with respect to the control. The study was carried out

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

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

Table 1: Antibiogram of *Staphylococcus saprophyticus*: Effect of biofield treatment on antimicrobial susceptibility.

S. No.	Antimicrobial	Gr. I	Gr. II		Gr. III (Day 10)
			Day 5	Day 10	
1.	Amoxicillin/k-clavulanate	≤4/2	≤4/2	>4/2	≤4/2
2.	Ampicillin/sulbactam	≤8/4	≤8/4	>16/8	≤8/4
3.	Ampicillin	≤0.25	≤0.25	4	≤0.25
4.	Azithromycin	≤2	≤2	≤2	≤2
5.	Cefazolin	≤8	≤8	16	≤8
6.	Cefepime	≤8	≤8	≤8	≤8
7.	Cefotaxime	≤8	≤8	≤8	≤8
8.	Ceftriaxone	≤8	≤8	≤8	≤8
9.	Cephalothin	≤8	≤8	≤8	≤8
10.	Chloramphenicol	≤8	≤8	≤8	≤8
11.	Ciprofloxacin	≤1	≤1	≤1	≤1
12.	Clindamycin	≤0.5	≤0.5	>2	≤0.5
13.	Erythromycin	≤0.5	≤0.5	≤0.5	≤0.5
14.	Gatifloxacin	≤2	≤2	≤2	≤2
15.	Gentamicin	≤4	≤4	≤4	≤4
16.	Imipenem	≤4	≤4	>8	≤4
17.	Levofloxacin	≤2	≤2	≤2	≤2
18.	Linezolid	≤2	≤2	>4	≤2
19.	Moxifloxacin	≤2	≤2	≤2	≤2
20.	Nitrofurantoin	≤32	≤32	≤32	≤32
21.	Norfloxacin	≤4	≤4	≤4	≤4
22.	Ofloxacin	≤2	≤2	≤2	≤2
23.	Oxacillin	≤0.25	≤0.25	2	≤0.25
24.	Penicillin	0.12	0.12	>8	0.12
25.	Piperacillin/tazobactam	≤4	≤4	-	≤4
26.	Rifampin	≤1	≤1	≤1	≤1
27.	Synercid	≤1	≤1	>2	≤1
28.	Tetracycline	≤4	≤4	≤4	≤4
29.	Trimethoprim/sulfamethoxazole	≤2/38	≤2/38	≤2/38	≤2/38
30.	Vancomycin	≤2	≤2	>16	≤2

MIC data are presented in µg/mL; Gr: Group; -, Data not available

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

with twenty-eight antimicrobials for assessment of susceptibility assay and thirty antimicrobials for estimation of MIC. Several antimicrobials viz. amoxicillin/k-clavulanate, ampicillin/sulbactam, cefazolin, cefepime, cefotaxime, ceftriaxone, cephalothin, clindamycin, imipenem, oxacillin, synercid and vancomycin were converted from susceptible (S) to resistance (R) in revived treated strain of *S. saprophyticus* (Gr. II) on day 10, while S in Gr. II, on day 5 and in Gr. III on day 10 as compared to the control (Gr. I). Moreover, ampicillin and penicillin were changed the sensitivity pattern from S to β-lactamase positive (BLAC) in Gr. II on day 10, while remained same i.e., S in Gr. II, on day 5 and in lyophilized treated cells (Gr. III) on day 10 with respect to the untreated group. Based on the literature, the species *S. saprophyticus* has the ability to produce BLAC or penicillinase enzyme that breakdowns the β-lactam ring present in penem heteronucleus [21]. In this experiment, the enzyme production ability might be enhanced after biofield energy treatment in revived treated cells. Hence, these two penem containing antibiotics showed BLAC response in Gr. II that was susceptible in the control sample. In this experiment, authors have found that after biofield treatment the organism could be able to produce β-lactamase enzyme that breaks the β-lactam ring present in either penems or cepheems nucleus. Overall, the treated cells of *S. saprophyticus* showed a significant (50%) alteration (fourteen out of twenty-eight) in antimicrobial sensitivity pattern in the revived treated Gr. II on day 10 as compared with the control. Fourteen, out of twenty-eight tested antimicrobials did not show any alteration in the

sensitivity profile of the treated cells of *S. saprophyticus*. The MIC values had revealed that various antibiotics such as ampicillin/sulbactam, cefazolin, imipenem, linezolid, and synercid were altered by two-fold in the revived treated group as compared to the control. Moreover, the MIC values of vancomycin and oxacillin were changed after biofield energy treatment by eight-fold in Gr. II on day 10 as compared to the control. The alterations in MIC values were observed by several-fold in ampicillin, clindamycin, oxacillin, and penicillin, in Gr. II on day 10 as compared to the control. Amoxicillin/k-clavulanate showed slight alteration in MIC in Gr. II on day 10 while unaltered in rest of treated groups with respect to the control. Overall, 36.67% (eleven out of thirty) antimicrobials exhibited an alteration in MIC value in revived treated cells (Gr. II, day 10) and rest of the antimicrobials did not report any change in MIC values in all the treated groups as compared to the control (Table 2). To achieve a strain-specific targeted drug therapy for UTIs, *in vitro* patient-specific data are necessary because, the data had provided wide geographical variability [22-24]. The production of β-lactamase is common among staphylococci and is the prime mechanism of penicillin resistance by these organisms. However, some researchers unable to find the enzyme that are responsible for resistance among the strain of *S. saprophyticus* [25,26].

Biochemical reactions studies

The study of biochemical reactions are the test battery for phenotypic identification of coagulase-negative staphylococci (CoNS). The specific biochemical that showed some changes against *S. saprophyticus* after biofield treatment as shown in Table 3. Based on

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

-: Negative; +: Positive; Gr: Group; [†]PGR: p-nitro phenyl β-D- glucuronide; [#]PGT: p-nitro phenyl β-D-galactopyranoside

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

Feature	Gr. I	Gr. II		Gr. III Day 10
		Day 5	Day 10	
Biotype	242076	246076	342064	242066
Organism Identification	<i>Staphylococcus saprophyticus</i>	<i>Staphylococcus saprophyticus</i>	<i>Staphylococcus hominis</i> subsp. <i>novobiosepticus</i>	<i>Staphylococcus saprophyticus</i>

Gr: Group

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

literature, for identification of CoNS it is necessary to perform initially the fermentation activities of xylose, sucrose, trehalose, maltose and mannitol, hemolysin production and anaerobic growth in thioglycollate [27]. In this experiment, the study results showed the positive (+) reactions of mannitol (MAN) and trehalose (TRE) that preliminary supported the control strain of *S. saprophyticus*. Based on these findings, rest of the biochemical reactions were performed including urease production, nitrate reduction, β -ornithine decarboxylation, and novobiocin resistance. However, after biofield energy treatment on *S. saprophyticus* the positive (+) reaction of MAN was converted to negative (-) in Gr. II and III on day 10 and TRE was converted to negative (-) in Gr. II on day 10 only as compared to the control. Several researchers reported that for the identification of *S. saprophyticus* in urine samples urease and novobiocin are the feasible alternative in support of this organism [28,29]. Here, the positive (+) reactions of urea (URE) and novobiocin (NOV) were matched with literature data. However, the positive (+) reactions of URE and NOV did not alter in all the treated groups that indicated that the basis phenotypic characteristics were not affected by biofield energy treatment on *S. saprophyticus*. Apart from these, the reduction of nitrate (NIT) is also another characteristics biochemical reaction for the identification of CoNS [30]. In this experiment, the negative (-) reaction of NIT indicated the reduction reaction in control sample that was correlated with the literature data. However, the conversion of negative (-) to positive (+) reaction of NIT in Gr. II on day 10 could be due to change in enzymatic reaction occurred after biofield energy treatment. If the bacterium has the ability to ferment butanediol then it showed positive reaction. It was described previously that the organism *S. saprophyticus* can ferment only xylose, sucrose, trehalose, maltose and mannitol. Hence, the negative (-) reaction of Voges-Proskauer (VP) was well supported with the literature data. However, the conversion to positive (+) reaction of VP in Gr. II on day 5 may be due to the effect of biofield energy treatment. These alterations of series of biochemical reactions occur due to change in enzymatic or metabolic activities after biofield treatment. Overall, 14.81% biochemical reactions were altered in tested twenty-seven biochemicals with respect to the control after biofield treatment. Rest of the biochemicals, did not show any change in all the treated groups after biofield treatment as compared to the control.

Identification of organism by biotype number

The species (*S. saprophyticus*) was identified based on the variety of conventional biochemical characters and biotyping. In this experiment, biotyping was performed using automated systems, and the results found significant changes in the biofield treated Gr. II (on day 5) and Gr. III (on day 10). Based on the biochemical results, biotype number was changed in the treated Gr. II on day 5 (246076, *Staphylococcus saprophyticus*), on day 10 (342064, *Staphylococcus hominis* subsp. *novobiosepticus*), and Gr. III on day 10 (242066, *Staphylococcus saprophyticus*) with respect to the control (242076) i.e., *S. saprophyticus* (Table 4). Biofield treatment might be responsible to do alteration in microorganism at enzymatic and/or genetic level, which may act on receptor protein [31]. Biofield treatment might induce significant changes in revived strain of *S. saprophyticus* and altered antimicrobials susceptibility pattern, MIC values, biochemical reactions, and ultimately change the biotype

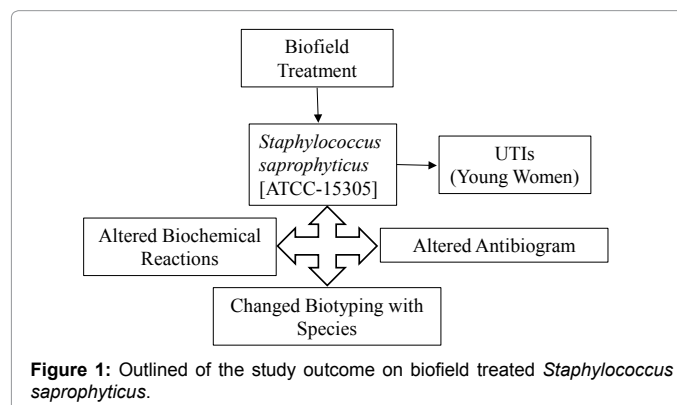


Figure 1: Outlined of the study outcome on biofield treated *Staphylococcus saprophyticus*.

number of microorganism (Figure 1). The microbe that was susceptible in control sample converted into resistant/BLAC in lyophilized treated cells of *S. saprophyticus* after biofield energy treatment. Based on these results, it is postulated that, biofield treatment has the ability to alter the sensitivity pattern of antimicrobials.

Conclusions

Altogether, the biofield treatment has significantly (50%) altered the susceptibility pattern with changed MIC values (36.67%) of tested antimicrobials against the biofield treated strain of *S. saprophyticus*. It also altered 14.81% biochemical reactions pattern and biotype number of biofield treated strain of *S. saprophyticus*. The biotype number with new species was identified in revived treated cells as *Staphylococcus hominis* subsp. *novobiosepticus*; (342064) with respect to the control i.e., *S. saprophyticus* (242076). Mr. Trivedi's biofield treatment could be applied as an alternative therapeutic approach against *S. saprophyticus*.

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