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Effect of Biofield Treatment on Phenotypic and Genotypic Characteristic of *Provindencia rettgeri*

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Abstract

Providencia rettgeri (*P. rettgeri*) is a clinically significant Gram-negative bacterium of genus *Providencia*, and commonly associated with hospital-acquired infection like urinary tract infection (UTI), gastroenteritis, and ocular infections. Present study was designed to evaluate the effect of biofield treatment on *P. rettgeri* against antimicrobial susceptibility, biochemical reaction pattern, biotype number, and 16S rDNA sequence. The samples of *P. rettgeri* (ATCC 9250) were divided into three groups: Gr.I (control), Gr.II (treatment, revived), and Gr.III (treatment, lyophilized). The Gr.II and III were treated with Mr. Trivedi's biofield, and then subsequently characterized for antimicrobial susceptibility, minimum inhibitory concentration (MIC), biochemical reactions, and biotype numbering. The 16S rDNA sequencing was carried out to correlate the phylogenetic relationship of *P. rettgeri* with other bacterial species. The treated cells of *P. rettgeri* showed an alteration in susceptibility of about 50% and 53.3% tested antimicrobials of Gr.II on day 5 and 10, respectively; and 53.3% of tested antimicrobials of Gr.III on day 10. MIC results showed a significant decrease in MIC values of 53.1, 56.3, and 56.3% antimicrobials in Gr.II on day 5, Gr.II on day 10, and Gr.III on day 10, respectively, as compared to control. The significant changes in biochemical reactions and biotype numbers were also observed in all the treated groups of *P. rettgeri*. Based on nucleotides homology and phylogenetic analysis the *P. rettgeri* was found to be *Proteus mirabilis* (GenBank Accession Number: AY820623) and nearest homolog species was found to be *Proteus vulgaris* (Accession No. DQ499636). These findings suggest that biofield treatment can prevent the emergence of absolute resistance of existing antimicrobials to *P. rettgeri*.

Keywords: *Providencia rettgeri*; Biofield treatment; Antimicrobial susceptibility; Biotype; 16S rDNA sequencing

Introduction

Presently, several microbes have been acquired the resistance to number of antimicrobial agents that were successfully treat the microbial infections earlier. The antimicrobial resistant microbes whether bacteria, fungi, viruses or parasites can survive in regular antimicrobial drugs therapy. The frequent and improper use and misuse of antimicrobial drugs accelerate the emergence of drug-resistant microbes, which were further spread by poor sanitary conditions and meager infection control [1]. Antimicrobial drugs prescribed in nearly all *Providencia* infections caused by five species: *Providencia rettgeri*, *P. alcalifaciens, P. rustigianii, P. stuartii,* and *P. heimbachae*. The *P. rettgeri* is a clinically significant, urease-producing, Gram-negative *Bacillus* and usually found in both water and land atmospheres. It is generally associated with opportunistic infections in humans such as traveler's diarrhea, urinary tract infections (UTI), skin infection, gastroenteritis, conjunctivitis, and endophthalmitis. The occurrence of *P. rettgeri* infection is common throughout the world with 6–33% of mortality rate, which is even greater in polymicrobial infection [2,3]. Recently, *P. rettgeri* has acquired antimicrobial resistance due to producing β-lactamase enzymes [4,5]. Therefore, due to the clinical significance of *P. rettgeri*, development of effective antimicrobial therapy is very needful for human health. As such, no medication is available to cure the resistant strain of microbe but an alternative approach known as biofield treatment is recently reported to alter the antimicrobial sensitivity in different microorganism [6].

The law of mass-energy inter-conversion is existed in the literature for more than 300 years, and the thought was initially reported by Hasenohrl followed by Einstein [7,8]. However, the conversion of mass into energy is well established, but its inversion *i.e.,* energy into mass has not yet proven scientifically. Furthermore, the energy can exists in several forms such as kinetic, potential, electrical, magnetic, and nuclear. Similarly, the human nervous system consists the energy in the form of electrical signals [9,10]. Thus, human has the ability to harness the energy from environment or universe and can transmit into any leaving 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. Whenever these electrical signals fluctuate with time, the magnetic field generates as per the Ampere-Maxwell law, and cumulatively known as electromagnetic field. Hence, the electromagnetic field being generated from the human body is known as biofield [11]. Mr. Mahendra Trivedi's biofield treatment has shown to transform the characteristics non-living and living things in several fields such as material science [12–17], agriculture [18–20], and biotechnology [21,22]. The biofield treatment has considerably altered the sensitivity of antimicrobials to some microbes [6,23,24].

By conceiving the challenges of antimicrobial resistance in *P. rettgeri*, and advantages of biofield treatment; this work was undertaken to evaluate the effects of biofield treatment on antimicrobials sensitivity, biotype number based on various biochemical reactions, and 16S rDNA gene sequencing of *P. rettgeri*.

Materials and Methods

The sample vial of *P. rettgeri* [American Type Culture Collection (ATCC) 9250] was procured from MicroBioLogics, Inc., USA, and

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stored as per the suggested storage conditions until further use. The antimicrobial susceptibility, biochemical reactions, and biotype number were evaluated on MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA) using Negative Breakpoint Combo 30 (NBPC30) panel. The 16S rDNA sequencing study was carried out in Gr. III sample using Ultrapure Genomic DNA Prep Kit; Cat KT 83 (Bangalore Genei, India).

Biofield treatment

The samples of *P. rettgeri* was divided in three groups: Gr.I (control), Gr.II (treatment, revived), and Gr.III (treatment, lyophilized). Subsequently, the treatment groups (Gr. II and III) were received biofield treatment. 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. Treated samples were assessed for antimicrobial sensitivity, biochemical reactions, and biotyping of *P. rettgeri*. The assays for Gr.II were assessed on day 5 and 10, and Gr.III was assessed on day 10. The 16S rDNA gene sequencing of *P. rettgeri* was also carried out.

Evaluation of antimicrobial susceptibility of *P. rettgeri*

Investigation of antimicrobial sensitivity of *P. rettgeri* was carried out with the help of automated instrument, MicroScan Walk-Away® using Negative Breakpoint Combo 30 (NBPC30) panel, as per the manufacturer's instructions [25]. The minimum inhibitory concentration (MIC) and a qualitative susceptibility like resistant (R), intermediate (I), susceptible (S), or inducible β-lactamases (IB) were determined by observing the lowest antimicrobial concentration showing growth inhibition [26]. The antimicrobial sensitivity study was carried out using following antimicrobials like amikacin, amoxicillin/K-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, cefotetan, cefoxitin, ceftazidime, cefuroxime, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gatifloxacin, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, nitrofurantoin, norfloxacin, piperacillin, piperacillin/tazobactam, tetracycline, ticarcillin, tobramycin, and trimethoprim/sulfamethoxazole. All these antimicrobials were purchased from Sigma-Aldrich, USA.

Biochemical studies

The biochemical studies of *P. rettgeri* were performed on MicroScan Walk-Away® [27,28]. Biochemical reactions patterns were carried out using 32 biochemicals *viz.* acetamide, adonitol, arabinose, arginine, cetrimide, cephalothin, citrate, colistin, esculin hydrolysis, nitrofurantoin, glucose, hydrogen sulfide, indole, inositol, kanamycin, lysine, malonate, melibiose, nitrate, oxidation-fermentation media, galactosidase, ornithine, oxidase, raffinose, Rhamnose, sorbitol, sucrose, tartarate, tryptophan deaminase, tobramycin, urea, and Voges-Proskauer. All these biochemical were procured from Sigma-Aldrich, USA.

Biotype number

The biotype numbers of *P. rettgeri* was determined by automated MicroScan Walk-Away® processed panel data utilizing biochemical reactions [25].

Amplification and gene sequencing of 16S rDNA

Genomic DNA was isolated and purified from treated group of *P. rettgeri* cells by using genomic purification Kit, as per the manufacturer's instructions. The 16S rDNA gene (~1.5 kb) was amplified employing universal primers forward 5'-AGAGTTTGATCCTGGC-3' and reverse 5'-GGTTACCTTGTTACGACTT-3'. After that, the amplified products were subjected to gel electrophoresis on 1.0% agarose gel, stained with ethidium bromide, and visualized under UV light in a gel documentation unit (BioRad Laboratories, USA). The amplified fragment of PCR was purified from the agarose gel by DNA Gel Extraction Kit. Sequencing of amplified product was carried out on commercial basis from Bangalore Genei, India. The obtained 16S rDNA sequences data were aligned and compared with the sequences, available in Gene Bank database of National Center for Biotechnology Information (NCBI) using the algorithm BLASTn program. The multiple sequence alignment/phylogenetic tree were constructed using MEGA 3.1 software using neighbour joining method [29].

Results

Evaluation of antimicrobial susceptibility

The results of biofield treatment on *P. rettgeri* in relation to sensitivity pattern and MIC of tested antimicrobials are summarized in Table 1 and 2, respectively. The biofield treated cells of *P. rettgeri* exhibited an alteration in susceptibility of 50% and 53.3% of total antimicrobials in Gr.II on day 5 and 10, respectively; and alteration of 53.3% of total antimicrobials in Gr.III on 10th day, with about 2–4 folds decrease in the MIC values of respective antimicrobials. Briefly, amikacin, cefepime, chloramphenicol, gentamicin, and tobramycin were converted from

S. No.	Antimicrobial	Gr.I Control	Gr.II day 5	Gr.II day 10	Gr.III day 10
1	Amikacin	R	S	S	S
2	Amoxicillin/K-clavulanate	IB	IB	IB	IB
3	Ampicillin/Sulbactam	I	IB	IB	IB
4	Ampicillin	R	R	I	IB
5	Aztreonam	R	IB	IB	IB
6	Cefazolin	I	IB	IB	IB
7	Cefepime	R	S	S	S
8	Cefotaxime	R	IB	IB	IB
9	Cefotetan	R	IB	IB	IB
10	Cefoxitin	R	IB	IB	IB
11	Ceftazidime	R	IB	IB	IB
12	Cefuroxime	R	IB	IB	IB
13	Ceftriaxone	IB	IB	IB	IB
14	Cephalothin	R	IB	IB	IB
15	Chloramphenicol	R	S	S	S
16	Ciprofloxacin	S	S	S	S
17	Gatifloxacin	S	S	S	S
18	Gentamicin	R	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	R	R	R	R
24	Norfloxacin	S	S	S	S
25	Piperacillin	IB	IB	IB	IB
26	Piperacillin/Tazobactam	IB	IB	IB	IB
27	Tetracycline	R	R	R	R
28	Ticarcillin	I	IB	IB	IB
29	Tobramycin	R	S	S	S
30	Trimethoprim/Sulfamethoxazole	S	S	S	S

Gr: Group; I: Intermediate; S: Susceptible; R: Resistant; IB: Reduced Activity of Inducible β-lactamase

Table 1: Effect of biofield treatment on *Providencia rettgeri* to susceptibility pattern of selected antimicrobials.

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

Gr: Group; MIC data are presented in µg/mL; ESBL-a, b Scrn: Extended-Spectrum β-Lactamase Screen

Table 2: Effect of biofield treatment on minimum inhibitory concentration (MIC) of *Providencia rettgeri*.

resistant (control) to susceptible in treated groups (Gr.II and Gr.III in all assessment). Similarly, cefotetan, cefoxitin, ceftazidime, cefuroxime, cefotaxime, cephalothin, and aztreonam were changed from resistant to inducible β-lactamase in entire treated groups. The sensitivity of ampicillin was altered from resistant to intermediate and inducible β-lactamase in Gr.II and III, respectively on day 10. Further, the ampicillin/sulbactam, cefazolin, and ticarcillin were converted from intermediate to inducible β-lactamase in all the treated groups. The MIC of all the above-mentioned antimicrobials were decreased about 2-folds except the ticarcillin and cefotaxime that showed about 4-folds decrease in MIC value.

Organism identification by biochemical reactions

The biochemical reactions of *P. rettgeri* are reported in Table 3, revealed an alteration in biochemical reaction pattern as 12.1% of total biochemicals in Gr.II on day 5 and 10, and 48.5% of total biochemicals in Gr.III on day 10. Briefly, the cephalothin, kanamycin, and tobramycin biochemical reactions were converted from positive to negative reaction in entire treated groups (Gr.II on day 5 and 10 and Gr.III on day 10). Biochemicals such as arabinose, hydrogen sulfide, lysine, malonate, melibiose, galactosidase, ornithine, raffinose, Rhamnose, sorbitol, sucrose, and Voges-Proskauer were changed from positive to negative reaction only in Gr.III on day 10 with respect to control. Further, tartarate was converted from negative to positive reaction in Gr.II on day 5 only, and tryptophan was converted from negative to positive in Gr.II and Gr.III on day 10, as compared to control.

Effect of biofield treatment on biotype number

The biotype numbers of *P. rettgeri* was determined on MicroScan Walk-Away® processed panel, using biochemical reaction data. The result exhibited alteration in biotype number of *P. rettgeri* in the entire treated groups (on all assessment day) as compared to control (Table 4).

16S rDNA gene sequencing

The 16S rDNA sequence was determined in *P. rettgeri*. The

S. No.	Code	Biochemical	Gr.I Control	Gr.II day 5	Gr.II day 10	Gr.III 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	Tartarate	-	+	-	-
30	TDA	Tryptophan deaminase	-	-	+	+
31	TO4	Tobramycin	+	-	-	-
32	URE	Urea	+	+	+	+
33	VP	Voges-Proskauer	+	+	+	-

Gr: Group; - (Negative); + (Positive)

Table 3: Effect of biofield treatment on *Providencia rettgeri* to biochemical reactions.

Feature	Gr.I Control	Gr.II day 5	Gr.II day 10	Gr.III day 10
Biotype	7776 5376	7776 5374	7776 5774	4064 0644
Organism Identification Name	*P. rettgeri*	*P. rettgeri*	*P. rettgeri*	*P. rettgeri*

Gr: Group

Table 4: Effect of biofield treatment on *Providencia rettgeri* to biotype.

alignment and assessment of the gene sequences data were performed by comparing with the sequences available in gene bank database of NCBI, using the algorithm BLASTn program. The phylogenetic tree was constituted using BLAST-Webpage (NCBI). Based on nucleotides homology and phylogenetic analysis, the Sample 3A (*P. rettgeri*) showed the genetic similarity with *Proteus mirabilis* (GenBank Accession Number: AY820623) with 100% identity of gene sequencing data. Ten different related bacterial species and *P. rettgeri* were considered as Operational Taxonomic Unites (OTUs) in order to investigate the phylogenetic relationship of *P. rettgeri* among other ten related species (Figure 1). Total 1495 base nucleotide of 16S rDNA gene sequences were analysed by multiple alignments using ClustalW of MEGA3.1 program [29]. Numbers of base substitutions per site from pairwise distance analysis between sequences (11 sequences) are shown in Table 5. Based on the phylogenetic tree and 16S rDNA sequencing, the nearest homolog genus-species of *P. rettgeri* was found to be *Proteus vulgaris* (Accession No. DQ499636). Some other close homologs of *P. rettgeri* can be found from the alignment as indicated in Table 5.

Discussion

Discovery of antimicrobial was a turning point in human history that revolutionized medication in several aspects, and saved the countless lives so far. Unfortunately, these wonder drugs have been accompanied by the quick emergence of resistant microbes. The extended spectrum ß-lactam (ESBL) antibiotics were widely used to cure the severe Gram-negative infections but due to production of extended spectrum β-lactamases (ESBLs) in the microorganism these ESBL antibiotics are now almost ineffective [30,31]. Similarly, the *P. rettgeri* has also acquired the antimicrobial resistance due to producing of β-lactamase enzyme and become a considerable threat to the human beings [4].

Research study suggests that most of the clinical isolates of *P. rettgeri* were found resistant to older cephalosporin, penicillin, fosfomycin and to antibiotics to which other Enterobacteriaceae species are also resistant [32]. Our experimental control sample (*P. rettgeri*) showed similar sensitivity and resistant pattern of tested antimicrobials. The treated sample of *P. rettgeri* exhibited the alteration in antimicrobial susceptibility from resistant to susceptible or inducible β-lactamases. The antimicrobials like amikacin, chloramphenicol, and gentamicin were converted from resistant (control) to susceptible with about 2-folds decrease in the MIC values. Likewise cefoxitin, ceftazidime, cephalothin, and aztreonam were converted from resistant to inducible β-lactamase, in entire treated groups with about 2-folds decrease in the MIC values. The highest decrees (*i.e.*, 4-folds) in MIC value were observed for cefotaxime and ticarcillin in the entire treated sample. Overall, different class of antimicrobials showed significant effect after

AN		1	2	3	4	5	6	7	8	9	10	11
DQ499636	1	—	0.981	0.993	0.964	0.992	0.963	0.991	0.951	0.948	0.992	0.991
DQ885259	2	0.019	—	0.983	0.963	0.983	0.962	0.982	0.957	0.953	0.985	0.982
AF008582	3	0.007	0.017	—	0.962	0.992	0.960	0.998	0.954	0.951	0.992	0.998
DQ205449	4	0.036	0.037	0.038	—	0.961	0.999	0.960	0.949	0.947	0.962	0.960
DQ885262	5	0.008	0.017	0.008	0.039	—	0.960	0.990	0.951	0.948	0.999	0.990
DQ205448	6	0.037	0.038	0.040	0.001	0.040	—	0.959	0.948	0.945	0.960	0.959
AY820623	7	0.009	0.018	0.002	0.040	0.010	0.041	—	0.952	0.948	0.991	1.000
AM040489	8	0.049	0.043	0.046	0.051	0.050	0.052	0.048	—	0.988	0.951	0.952
AM040490	9	0.052	0.047	0.050	0.053	0.052	0.055	0.052	0.013	—	0.948	0.948
DQ885257	10	0.008	0.015	0.008	0.038	0.001	0.040	0.009	0.050	0.052	—	0.991
Sample 3A	11	0.009	0.018	0.002	0.040	0.010	0.041	0.000	0.048	0.052	0.009	—

AN, GenBank Accession Number

Figure 1: Distance matrix based on nucleotide sequence homology (Using Kimura-2 Parameter).

Alignment View	ID	Alignment results	Sequence description
	3A	0.93	Sample studied
	AY820623	0.93	*Proteus mirabilis*
	AF008582	0.94	*Proteus mirabilis*
	DQ499636	0.94	*Proteus vulgaris*
	DQ885262	0.98	*Proteus hauseri* strain NCTC 4175
	DQ885257	0.98	*Proteus vulgaris* strain ATCC 29905
	DQ885259	0.92	*Proteus myxofaciens* strain NCIMB 13273
	DQ205449	0.9	*Xenorhabdus hominickii* strain KR05
	DQ205448	0.89	*Xenorhabdus hominickii* strain KR01
	AM040489	0.93	*Providencia rustigianii* type strain DSM 4541
	AM040490	0.88	*Providencia heimbachae* type strain DSM 3591

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

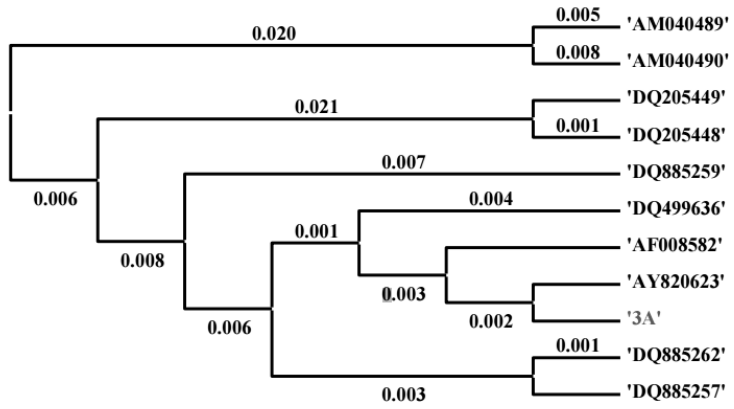

Figure 2: Phylogenetic tree of the partial 16S rDNA gene sequencing using MEGA 3.1 software by Neighbor joining method.

biofield treatment *viz.* β-Lactam penicillin (ampicillin/sulbactam), cephalosporin (cefazolin, cefepime, cefotetan, and cefuroxime), monobactum (azetronan), and aminoglycosides (tobramycin and amikacin). In addition, the treated sample of *P. rettgeri* also showed the considerable alteration in biochemical reactions patterns. The biotype number of *P. rettgeri* was also changed from 7776 5376 (control) to 7776 5374, 7776 5774, in Gr.II on day 5 and 10, respectively, and 4064 0644 in Gr.III on day 10 (Table 4). Based on the BLASTn analysis, the sample 3A was identified as *P. mirabilis* with 100% similarity in gene sequence. The phylogenetic tree diagram (Figure 2) anticipated the closest species of *P. rettgeri* to be as *Proteus vulgari.* The present study revealed that biofield treatment could alter the sensitivity of antimicrobials against *P. rettgeri*. Based on these results, it seems that biofield treatment can be a better alternate of existing drug therapy in future.

Conclusions

Altogether, these results suggest that Mr. Trivedi's biofield treatment has a significant impact on antimicrobial susceptibility, MIC value, biochemical reactions pattern, and biotype number of *P. rettgeri.*

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