Effect of Biofield Treatment on Phenotypic and Genotypic Characteristic of Providencia 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 Gr.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 Hasenohr 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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Received July 03, 2015; Accepted July 20, 2015; Published July 27, 2015

Citation: Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Effect of Biofield Treatment on Phenotypic and Genotypic Characteristic of Providencia rettgeri. Mol Biol 4: 129. doi:10.4172/2168-9547.1000129

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Biofield treatment

The samples of *P. rettgeri* were 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 handled 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 following antinicrobials like amikacin, amoxicillin/ K-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cepfime, 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® 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 following antinicrobials like amikacin, amoxicillin/ K-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cepfime, 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.

Biotype number

The biotype numbers of *P. rettgeri* were 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’-AGAGTTTTGATCCTGCGG-3’ and reverse 5’-GGTACCTTGTACCAGCTT-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 Geni, 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, cefpime, chloramphenicol, gentamicin, and tobramycin were converted from...
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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 cefalothin, kanamycin, and tobramycin biochemical reactions were converted from positive to negative reaction in entire treated groups (Gr.I 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, sucrase, 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
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 β-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, cefalothin, 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 | 0.962 | 0.962 | 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 | —     | 0.448 | 0.448 |
| 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).
biofield treatment viz. β-Lactam penicillin (ampicillin/sulbactam), cephalosporin (cefazolin, cefepime, cefotetan, and cefuroxime), monobactam (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 vulgaris. 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.

Acknowledgement

Authors gratefully acknowledged the whole team of PD Hinduja National Hospital and MRC, Mumbai, Microbiology Lab for their support.

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