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Characterization of Antimicrobial Susceptibility Profile of Biofield Treated Multidrug-resistant *Klebsiella oxytoca*

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Abstract

Klebsiella are opportunistic pathogens that cause a wide spectrum of severe diseases. The aim of the present study was to investigate the impact of biofield treatment on multidrug resistant strain of *K. oxytoca* with respect to antibiogram pattern along with biochemical study and biotype number. Clinical lab isolate of *K. oxytoca* was divided into two groups *i.e.* control and treated. Control group remain untreated and treated group was subjected to Mr. Trivedi's biofield. The analysis was done on day 10 after biofield treatment and compared with control group. Control and treated groups were analyzed for antimicrobial susceptibility pattern, minimum inhibitory concentration (MIC), biochemical reactions and biotype number using MicroScan Walk-Away® automated system. Experimental results showed the impact of biofield treatment on *K. oxytoca* and found alteration in both antimicrobial sensitivity and MIC values as compared with untreated group. Antimicrobial sensitivity of about 26.67% tested antimicrobials out of thirty was altered with respect to control. MIC results showed about 12.50% alterations in tested antimicrobials as compared to control. Biochemical study showed 24.24% alteration in tested biochemical reactions after biofield treatment. A significant change in biotype number (7713 5272) was identified after biofield treatment as compared to control (7775 4332). In treated group, a new species was identified as *Kluyvera ascorbata*, as compared to control, *K. oxytoca*. Study findings suggest that biofield treatment has a significant effect in altering the antimicrobial sensitivity, MIC values, biochemical reactions and biotype number of multidrug resistant strain of *K. oxytoca*. Biofield treatment could be applied to alter the antibiogram-resistogram pattern of antimicrobials.

Keywords: *Klebsiella oxytoca*; Multidrug resistant; Antibiogram; Biofield treatment; Biochemical reactions; Biotyping

Introduction

Klebsiella oxytoca (K. oxytoca) is a Gram-negative pathogen, cylindrical rod shaped, non-motile in nature, and belongs to *Enterobacteriaceae* family. *Klebsiella* spp. are ubiquitous in environment [1], but *K. oxytoca* can be cultured from intestines of healthy humans and animals, oropharynx, mucous membrane and skin. *K. oxytoca* initially named as *Aerobacter aerogens*, which was identified as *Klebsiella pneumoniae*, but recent report classified it as *K. oxytoca*, on the basis of indole-positive test and ability to grow on melezitose, not in 3-hydroxybutyrate [2]. It is considered as an opportunistic pathogen, as most of the cases *K. oxytoca*-infected persons remain asymptomatic. However, *K. oxytoca* is now recognized as important clinical pathogen in hospitalized patients causing major nosocomial infections in children and neonates [3]. It is reported in many etiological human infections such as urinary tract infection, septic arthritis, bacteremia, septicemia, cholecystitis, soft tissue infections, and most recently in colicky neonates [1, 4-7]. During last few years, incidence of extended spectrum β-lactamase producing multi-drug-resistance (MDR) *Klebsiella* spp. had increased. Cases of MDR infections had been increased suddenly, which resulted in ineffective antimicrobials treatment. Clinicians prefer multiple combination bactericidal therapy against infection instead of single drug. Recently, an alternate approach called biofield treatment on pathogenic microorganism is reported to alter the antimicrobial susceptibility.

Biofield is a cumulative outcome of electric and magnetic field energy, exerted by the human body. However, the energy can exist in several forms such as kinetic, potential, electrical, magnetic, and nuclear. Similarly, the human nervous system consists of the energy and chemical information in the form of electrical signals. Thus, 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 responding into useful way via biofield energy. Mr. Trivedi's unique biofield treatment is also known as The Trivedi Effect®. In spite of countless study reports on biofield therapies [8,9], there are very few well controlled and peer-reviewed experimental studies on pathogenic or MDR microbes. According to law of mass-energy inter-conversion [10], the conversion of mass into energy is well established, but its inversion *i.e.* energy into mass has not yet proved scientifically. Whenever these electrical signals fluctuate with time, the magnetic field generates as per the Ampere-Maxwell law, and cumulatively known as electromagnetic field. Mr. Trivedi's biofield treatment is well-known to change the various physicochemical characteristics of metals and ceramics [11-14]. In addition, his unique biofield treatment has considerably altered the antimicrobials susceptibility and biochemical reactions of pathogenic microbes [15-17]. In agricultural science, biofield treatment altered the growth, characteristics and yield of important medicinal plants [18-21]. On the basis of several reports on biofield treatment, present study was designed to study the impact of biofield on MDR isolate of *K. oxytoca*, for its antimicrobials susceptibility pattern, minimum

inhibitory concentration (MIC), along with biotyping based on variation in biochemical reactions.

Materials and Methods

Experimental design and biofield treatment

MDR clinical strain of *K. oxytoca* was collected from stored stock cultures of clinical sample in Microbiology Lab, Hinduja Hospital, Mumbai. MDR strain was divided in two groups *i.e.* control and treatment. Treatment group, in sealed pack was handed over to Mr. Trivedi for biofield treatment under laboratory conditions. Mr. Trivedi provided the treatment through his energy transmission process to the treated groups without touching the samples. The biofield treated sample was returned in the similar sealed condition for further analysis on day 10 with respect to control using the standard protocols. After biofield treatment, treated sample was analyzed for antimicrobial susceptibility, biochemical reactions and biotype number using MicroScan Walk-Away® (Dade Behring Inc., USA) and Negative Break Point Combo (NBPC 30) panel with respect to control groups. The antimicrobials and biochemicals were procured from Sigma Aldrich, MA, USA.

Evaluation of antimicrobial susceptibility assay

Antimicrobial susceptibility pattern of *K. oxytoca* was studied using MicroScan Walk-Away® NBPC 30 as per manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate, and R: Resistant) and MIC were determined by observing the lowest antimicrobial concentration showing growth inhibition [22].

Biochemical reaction study

Biochemical study of *K. oxytoca* was determined by MicroScan Walk-Away® system in both control and treated groups [22].

Identification by biotype number

The biotype number of *K. oxytoca* control and treated samples were determined by MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data [22].

Results and Discussion

Antimicrobial susceptibility test

Results of antimicrobial sensitivity pattern and MIC of *K. oxytoca* isolate are summarized in Tables 1 and 2 respectively.

S. No.	Antimicrobial	Control	Treated
1	Amikacin	S	R
2	Amoxicillin/k-clavulanate	I	R
3	Ampicillin/sulbactam	R	R
4	Ampicillin	R	R
5	Aztreonam	EBL	R
6	Cefazolin	R	R
7	Cefepime	R	R
8	Cefotaxime	EBL	R
9	Cefotetan	S	R
10	Cefoxitin	R	R
11	Ceftazidime	EBL	R
12	Ceftriaxone	EBL	R
13	Cefuroxime	R	R
14	Cephalothin	R	R
15	Chloramphenicol	R	R
16	Ciprofloxacin	R	R
17	ESBL-a Scrn	EBL	-
18	ESBL-b Scrn	EBL	-
19	Gatifloxacin	R	R
20	Gentamicin	R	R
21	Imipenem	S	S
22	Levofloxacin	R	R
23	Meropenem	S	S
24	Moxifloxacin	R	R
25	Piperacillin/tazobactam	S	I
26	Piperacillin	R	R
27	Tetracycline	R	R
28	Ticarcillin/k-clavulanate	R	R
29	Tobramycin	R	R
30	Trimethoprim/ sulfamethoxazole	R	R

R: Resistant; I: Intermediate; S: Susceptible; ESBL-a, b Srcn: Extended-spectrum-β-lactamase screen; EBL: Suspected extended-spectrum β-lactamases; -: Not tested

Table 1: *In-vitro* antimicrobial susceptibility assay of multidrug resistant *Klebsiella oxytoca*.

The biofield treatment on MDR strain of *K. oxytoca* showed a significant change in sensitivity pattern of different tested antimicrobials such as amikacin and cefotetan changed from sensitive (S) to resistance (R), while aztreonam, cefotaxime, ceftazidime, and ceftriaxone sensitivity were changed from suspected extended-spectrum β-lactamases to resistance. Moreover, amoxicillin/clavulanate sensitivity changed from intermediate to resistant while piperacillin/tazobactam was changed from susceptible to intermediate as compared to control. Overall 26.67% alteration was reported out of thirty tested antimicrobials after biofield treatment. Rest of the twenty-two antimicrobials did not show any change in sensitivity after biofield treatment compared to control. MIC results showed 12.5% alteration in tested antimicrobials after biofield treatment on MDR strain of *K. oxytoca*. MIC value in four antimicrobials was increased out of thirty-two tested antimicrobials. Amikacin and cefotetan showed about two-folds increase in MIC value (≤ 16 to >32 µg/mL) as compared to

control. Piperacillin/tazobactam showed four folds increase in MIC value (<16 to 64 µg/mL) as compared to control. Amoxicillin/k-clavulanate also showed increase MIC value with respect to control. Rest of the antimicrobials did not show any alteration in MIC values with respect to control (Table 3).

This study investigated the influence of biofield treatment on MDR strain of *K. oxytoca* with respect to antimicrobial sensitivity assay, and results found that biofield treatment has the potential to alter the sensitivity and MIC values of antimicrobials against biofield treated pathogen. The increased emergence of MDR strains of *Klebsiella* spp., in immunocompromised patients and increased infections lead to serious matter of concern worldwide. Extended spectrum β-lactamase (ESBL) producing species have still the serious problem worldwide, which may be due to continuous new drug discovery [2]. Results suggest the natural resistant pattern of MDR strain of *K. oxytoca* against most of the tested antimicrobials. Antimicrobial sensitivity of *K. oxytoca* is well supported with literature data [23]. Biofield treatment group showed significant effect on ESBL producing antimicrobials, as sensitivity after biofield treatment changed to resistant in case of aztreonam, cefotaxime, ceftazidime, and ceftriaxone. Most of the clinical strains of *K. oxytoca* produced chromosomal and plasmid mediated β-lactamase. Chromosomal mediated β-lactamases had the capacity to hydrolyze extended spectrum antimicrobials such as cephalosporin, and aztreonam. Mutational hyper production of β-lactamase results in a characteristic antibiogram with resistant pattern against piperacillin, cefuroxime, and aztreonam [24]. However, most of the clinical isolates of *K. oxytoca* have been associated with low production of β-lactamase. Biofield treatment might induce some enzymatic changes which result in significant alteration in antimicrobial sensitivity and MIC values. Resistant pattern in MDR is also associated with alteration in cell membrane, which may causes decrease uptake of antimicrobial, drug target enzyme overexpression, or alteration in drug efflux pump [25-27]. Biofield treatment in MDR *K. oxytoca* might alter the cell membrane permeability which results in alteration in sensitivity of tested antimicrobials.

S. No.	Antimicrobial	Control	Treated
1	Amikacin	≤ 16	>32
2	Amoxicillin/k-clavulanate	16/8	>16/8
3	Ampicillin/sulbactam	>16/8	>16/8
4	Ampicillin	>16	>16
5	Aztreonam	>16	>16
6	Cefazolin	>16	>16
7	Cefepime	>16	>16
8	Cefotaxime	>32	>32
9	Cefotetan	≤ 16	>32
10	Cefoxitin	>16	>16
11	Ceftazidime	>16	>16
12	Ceftriaxone	>32	>32
13	Cefuroxime	>16	>16
14	Cephalothin	>16	>16
15	Chloramphenicol	>16	>16
16	Ciprofloxacin	>2	>2
17	ESBL-a Scrn	>4	>4
18	ESBL-b Scrn	>1	>1
19	Gatifloxacin	>4	>4
20	Gentamicin	>8	>8
21	Imipenem	≤ 4	≤ 4
22	Levofloxacin	>4	>4
23	Meropenem	≤ 4	≤ 4
24	Moxifloxacin	>4	>4

25	Nitrofurantoin	>64	>64
26	Norfloxacin	>8	>8
27	Piperacillin/tazobactam	≤ 16	64
28	Piperacillin	>64	>64
29	Tetracycline	>8	>8
30	Ticarcillin/k-clavulanate	>64	>64
31	Tobramycin	>8	>8
32	Trimethoprim/sulfamethoxazole	>2/38	>2/38
MIC values are presented in µg/mL; ESBL-a,b Srcn: Extended-spectrum-β-lactamase screen			

Table 2: Minimum inhibitory concentration (MIC) tested antimicrobials of multidrug resistant *Klebsiella oxytoca*.

Identification of organism by biochemical reactions

Several phenotypic identification tests are available to differentiate the *Klebsiella* species. Experimental identification of *K. oxytoca* was performed using different standard biochemical reaction analysis. Adonitol, inositol, urea, and Voges-Proskauer showed negative reaction *i.e.* positive to negative, while cetrimide, citrate, hydrogen sulfide, and ornithine showed positive reaction *i.e.* negative to positive after biofield treatment as compared to control. Rest of the biochemical reactions were not altered after biofield treatment with respect to control. Overall, biochemical reactions showed the alteration of 24.24% after biofield treatment. Experimental control biochemical reaction data of *K. oxytoca* are well supported with literature data [2]. Biofield treatment showed a significant alteration in positive as well as negative reactions in tested biochemical, which are the basic characteristics of *K. oxytoca*. The standard positive biochemical reactions of *K. oxytoca* were reported in case of indole, lysine decarboxylase, L-sorbose, malonate, urea and Voges-Proskauer while negative reactions in ornithine decarboxylase, gas production, and citrate.

S. No.	Code	Biochemical	Control	Treated
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/glucose	+	+
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	Tartrate	-	-
30	TDA	Tryptophan deaminase	-	-
31	TO4	Tobramycin	+	+
32	URE	Urea	+	-
33	VP	Voges-Proskauer	+	-
- (negative); + (positive); ONPG: Ortho-nitrophenyl-β-galactoside				

Table 3: Biochemical identification of multidrug resistant *Klebsiella oxytoca*.

Identification of organism by biotype number

On the basis of above biochemical changes, biotyping was performed to check the identity of microorganism after biofield treatment using an automated system. Results of biotyping found a significant changed in biotype number (7713 5272) in treated group on day 10, with respect to control (7775 4332). The organism was identified as *Kluyvera ascorbata* in treated group after biofield treatment as compared to control organism, *K. oxytoca* (Table 4). Biofield treatment on pathogenic microorganism showed significant alteration in biochemical reactions followed by altered biotype number, which are well supported with literature reports [15-17].

Biofield therapies in biomedical health care system are very popular and reported to improve human well-being with respect to several diseased conditions [28]. Increased emergence of resistant microorganisms due to widespread uses of antibiotics contributed to the spread of multidrug resistant organisms [29]. Biofield treatment is practiced by many heath care professionals as it was accepted by National Center for Complementary and Alternative Medicine (NCCAM), in complementary and alternate medicine [30]. Biofield treatment in pathogenic microorganisms had been reported to alter the antimicrobial sensitivity, phenotypic characteristics, and growth of microorganism [16,17]. It results in altered sensitivity of antimicrobials which may involve cellular changes in biofield treated *K. oxytoca* at molecular and/or genetic level [31]. Results showed that, biofield treatment induced changes in susceptibility pattern of antimicrobials, MIC values biochemical reactions, and biotype number of MDR strain of *K. oxytoca*.

Feature	Control	Treated
Biotype	7775 4332	7713 5272
Organism Identification	*Klebsiella oxytoca*	Kluyvera ascorbata

Table 4: Effect of biofield treatment on multidrug resistant strain of *Klebsiella oxytoca* to its biotype number.

Conclusion

Altogether, the biofield treatment on MDR strain of *K. oxytoca* showed alteration of antimicrobial sensitivity pattern, MIC, biochemical reactions followed by biotype number. Altered biochemical reactions may be responsible for changed biotype number, and a new species was identified as *Kluyvera ascorbata*, as compared to control. Alteration in above standard microbiological techniques after biofield treatment might involve the changes at enzymatic or genetic level of *K. oxytoca*, which can be further studied at molecular level with respect to altered antimicrobial sensitivity and biotype number. Based on the study outcomes, biofield treatment could be applied to alter the sensitivity pattern of antimicrobials, against multidrug resistance strain of *K. oxytoca*.

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Conflict of interest

The authors declare that they have no competing interest.

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