

Antibiogram of Multidrug-Resistant Isolates of *Pseudomonas aeruginosa* after Biofield Treatment

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Abstract

In recent years, prevalence of multidrug resistance (MDR) in Pseudomonas aeruginosa (P. aeruginosa) has been noticed with high morbidity and mortality. Aim of the present study was to determine the impact of Mr. Trivedi's biofield treatment on MDR clinical lab isolates (LS) of P. aeruginosa. Five MDR clinical lab isolates (LS 22, LS 23, LS 38, LS 47, and LS 58) of P. aeruginosa were taken and divided into two groups i.e. control and biofield treated. Control and treated group were analyzed for antimicrobial susceptibility pattern, minimum inhibitory concentration (MIC), biochemical study and biotype number using MicroScan Walk-Away® system. The analysis was done on day 10 after biofield treatment as compared with control group. Antimicrobial sensitivity assay showed 60% alteration in sensitivity of tested antimicrobials in MDR isolates of P. aeruginosa after biofield treatment. MIC results showed an alteration in 42.85% tested antimicrobials out of twenty eight after biofield treatment in five isolates of MDR P. aeruginosa. Biochemical study showed a 48.48% change in tested biochemical reactions out of thirty three as compared to control. A significant change in biotype numbers was reported in three clinical lab isolates of MDR P. aeruginosa out of five, after biofield treatment as compared to respective control. On the basis of changed biotype number (7302 0052) in biofield treated LS 23, new organism was identified as Citrobacter freundii as compared to control (0206 3336). A very rare biotype number (7400 4263) was found in biofield treated LS 38, as compared to control (0206 3736). Study results suggest that biofield treatment on lab isolates of MDR P. aeruginosa has significant effect on the antimicrobial sensitivity, MIC values, biochemical reactions and biotype number. Biofield treatment might prevent the emergence of absolute resistance pattern of useful antimicrobials against MDR isolates of P. aeruginosa.

Keywords: *Pseudomonas aeruginosa*; Biofield treatment; Multidrugresistant; Antimicrobial susceptibility; Biochemical reaction; Biotyping

Introduction

Antimicrobial agents are widely used therapeutic option against infections caused by pathogenic microbes. However, through different strategies and mechanism, these microorganisms combat the effect of antimicrobial agents, as multidrug resistant (MDR) clinical strains are the best example world-wide. Pseudomonas aeruginosa (P. aeruginosa) is a ubiquitous, gram-negative bacterium and versatile opportunistic pathogen, associated with nosocomial infections along with other serious implications with high rate of morbidity and mortality [1]. Increasing resistant towards the available antimicrobials preclude the effectiveness of any antimicrobial regimen [2,3]. Because of increasing MDR P. aeruginosa isolates in health care setting, infections are difficult to treat, causing life threating conditions [4]. P. aeruginosa is one of the major pathogen related with hospital acquired infections especially in Intensive care unit [5]. According to the report of nosocomial infection surveillance system of center for disease control and prevention, P. aeruginosa is second most common cause of nosocomial pneumonia, third most common in nosocomial urinary tract infections and eighth most common cause of nosocomial bacteraemia [6]. MDR mechanism in P. aeruginosa are due to acquisition of resistance genes (β-lactamases) or because of aminoglycoside modifying enzymes [7], or due to chromosomal genes mutation involved against antimicrobials [8].

Despite of several advances in medical sciences, new generation antimicrobials against MDR strains of P. aeruginosa associated infections are still a serious challenge [9]. Recently, an alternate approach called biofield treatment is reported with effectively inhibiting the growth of bacterial cultures [10]. Biofield is the name given to the electromagnetic field/energy that permeates and surrounds living organisms. However, the energy can exists 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 that is called biofield energy and the process is known as biofield treatment. Few cases of biofield therapies are reported effectively [11,12], but very less controlled and experimental studies on biofield and electromagnetic fields treatment are practiced worldwide [13]. According to law of mass-energy inter-conversion [14], the conversion of mass into energy is well stabilized, but its inversion *i.e.* energy into mass has not yet proved scientifically. Whenever 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 physicochemical and atomic characteristics of various materials. Mr.

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Trivedi's biofield treatment had been studied and reported in altering the antimicrobial susceptibility and biochemical reactions of microbes against tested antimicrobials [15-17]. It has also significantly reported in field of material science [18-20]. Biofield treated crops had been reported for a significant change on growth, characteristics and yield of plants [21-24]. Present study reports the impact of biofield treatment on MDR isolates of *P. aeruginosa*, for its antimicrobial susceptibility pattern along with minimum inhibitory concentrations (MIC), biochemical reactions, and biotyping.

Materials and Methods

Experimental design and biofield treatment

MDR clinical lab isolates (*i.e.* LS 22, LS 23, LS 38, LS 47, and LS 58) of *P. aeruginosa* were obtained from stored stock cultures in Microbiology Lab, Hinduja Hospital, Mumbai. Each MDR lab isolates was divided into two groups *i.e.* control and treatment. Treatment group, 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 groups without touching the samples. The biofield treatment, following parameters like antimicrobial susceptibility, MIC, biochemical reactions, and biotype number were measured by MicroScan Walk-Away* (Dade Behring Inc., USA) with respect to control group. All antimicrobials and biochemicals were procured from Sigma-Aldrich, USA.

Evaluation of antimicrobial susceptibility assay

Antimicrobial susceptibility pattern of MDR lab isolates of P. aeruginosa was studied using MicroScan Walk-Away[®] using Negative Break Point Combo (NBPC 30) panel as per manufacturer's instructions. The antimicrobial susceptibility pattern (S: Susceptible, I: Intermediate, IB: Inducible β-lactamase; and R: Resistant) and MIC values were determined by observing the lowest antimicrobial concentration showing growth inhibition [25]. The antimicrobials used in the susceptibility assay viz. amikacin, amoxicillin/K-clavulanate, ampicillin/sulbactam, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, cefotetan, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, nitrofurantoin, gentamicin, imipenem, levofloxacin, meropenem, norfloxacin, piperacillin, piperacillin/tazobactam, tetracycline, ticarcillin/kclavulanate, tobramycin, and trimethoprim/sulfamethoxazole.

Biochemical study

Biochemical studies of MDR isolates of *P. aeruginosa* were determined by MicroScan Walk-Away^{*} using NBPC 30 panel system in both control and treated groups [25]. Biochemicals used in the study are acetamide, adonitol, arabinose, arginine, cetrimide, cephalothin, citrate, colistin, esculin hydrolysis, nitrofurantoin, glucose, hydrogen sulfide, indole, inositol, kanamycin, lysine, malonate, melibiose, nitrate, oxidation-fermentation, galactosidase, ornithine, oxidase, penicillin, raffinose, rhamnose, sorbitol, sucrose, tartrate, tryptophan deaminase, tobramycin, urea, and Voges-Proskauer.

Identification by biotype number

The biotype number of MDR isolates of *P. aeruginosa* in control and treated sample were determined followed by identification of microorganism by MicroScan Walk-Away[®] processed panel data report with the help of biochemical reaction data [25].

Results and Discussion

Antimicrobial susceptibility pattern

The sensitivity pattern of MDR isolates of *P. aeruginosa* against antipseudomonal antimicrobials agents are demonstrated in Table 1. All these changes were observed on day 10 after biofield treatment as compared to control group. Overall, 60% of tested fifteen antimicrobials, showed an alteration in antimicrobial sensitivity pattern after biofield treatment in MDR isolates of P. aeruginosa. Aztreonam (R \Rightarrow I in LS 22), cefepime (R \Rightarrow S in LS 23 and R \Rightarrow I in LS 47), imipenem (R \rightarrow I in LS 58) and meropenem (R \rightarrow S in LS 23 and R \rightarrow I in LS 58) showed increased antimicrobial sensitivity pattern after biofield treatment in clinical isolates of P. aeruginosa. Above antimicrobials also showed decrease in MIC values in treated group (Table 2). Sensitivity pattern of aztreonam, ceftazidime, and ceftriaxone antimicrobials changed from $R \rightarrow IB$, while piperacillin changed from IB \rightarrow R and ticarcillin/K-clavulanate changed from IB \rightarrow I in LS 23. Antimicrobial sensitivity pattern of biofield treated LS 38 was changed from IB \rightarrow R in case of ceftazidime, piperacillin, piperacillin/tazobactam, and ticarcillin/K-clavulanate, and in case of aztreonam and ceftriaxone sensitivity changed from $I \rightarrow R$, while $S \rightarrow R$, in case of cefepime. Biofield treated LS 47 showed changed sensitivity pattern of $R \rightarrow IB$ in piperacillin/tazobactam and $IB \rightarrow R$ in ticarcillin/K-clavulanate antimicrobials. Piperacillin showed changed antimicrobial sensitivity pattern from $R \rightarrow IB$ in biofield treated LS 58. Amikacin, cefotaxime, ciprofloxacin, gentamicin, levofloxacin, and tobramycin antimicrobials did not show any change in sensitivity pattern in biofield treated clinical isolates of P. aeruginosa.

Antimicrobial	LS	22	LS	23	LS	38	LS	47	LS	58
	С	т	с	т	с	т	С	т	с	т
Amikacin	R	R	s	s	R	R	R	R	R	R
Aztreonam	R	I	R	IB	I	R	R	R	R	R
Cefepime	R	R	R	s	s	R	R	I	R	R
Cefotaxime	R	R	R	R	R	R	R	R	R	R
Ceftazidime	R	R	R	IB	IB	R	R	R	R	R
Ceftriaxone	R	R	R	IB	1	R	R	R	R	R
Ciprofloxacin	R	R	R	R	R	R	R	R	R	R
Gentamicin	R	R	R	R	R	R	R	R	R	R
Imipenem	R	R	s	s	s	s	s	s	R	I
Levofloxacin	R	R	R	R	R	R	R	R	R	R
Meropenem	R	R	R	s	s	s	S	s	R	I
Piperacillin	R	R	IB	R	IB	R	R	R	R	IB
Piperacillin/tazobactam	R	R	IB	IB	IB	R	R	IB	IB	IB

Ticarcillin/k-clavulanate	R	R	IB	I	IB	R	IB	R	R	R
Tobramycin	R	R	R	R	R	R	R	R	R	R
C: Control; T: Treatment; I Susceptible; IB: Inducible β-I				e; R:	Resi	stant	; I: I	nterm	ediate	ə; S:

 Table 1: Effect of biofield treatment on antimicrobial susceptibility of multidrug resistant isolates of *Pseudomonas aeruginosa*.

Experimental results of antimicrobial sensitivity assay showed altered sensitivity pattern in biofield treated clinical isolates of *P. aeruginosa*. Increasing antimicrobial resistance against various antipseudomonal drugs in clinical isolates, has been reported world-wide [26,27] which leads to a serious therapeutic problem. The resistance profiles of *P. aeruginosa* showed varying degrees of resistance to imipenem in recent years [28]. Obritsch *et al.* defined resistance pattern of MDR *P. aeruginosa* against β -lactam (piperacillin, piperacillin/tazobactam, cefepime, ceftazidime, and ticarcillin), carbapenems (imipenem, and meropenem), aminoglycosides (gentamycin, tobramycin, and amikacin) and fluoroquinolone (ciprofloxacin). Antimicrobial sensitivity results of control MDR isolates of *P. aeruginosa* were well supported with literature data [29]. Biofield treatment might be alternative approach to increase the sensitivity of antimicrobials. In case of aztreonam, cefepime,

imipenem, and meropenem, antimicrobial sensitivity was improved after biofield treatment in clinical MDR isolates.

Determination of minimum inhibitory concentration

MIC values of all the clinical MDR isolates of control and biofield treated P. aeruginosa are summarized in Table 2. Overall, 13 out of 28 tested antimicrobial (42.85%) reported with altered MIC values after biofield energy treatment in clinical MDR isolates of P. aerugonisa. MIC values were decreased four fold in ceftriaxone (less than equal to 8 µg/ml) and two fold in six antimicrobials *i.e.* aztreonam, cefepime, cefotetan, ceftazidime, chloramphenicol, and meropenem after biofield treatment in LS 23 isolate. Other isolates were also showed decreased MIC values in tested antimicrobials such as aztreonam (16 µg/ml in LS 22), cefepime (16 µg/ml in LS 47), imipenem (8 µg/ml in LS 58), meropenem (8 µg/ml in LS 58), nitrofurantoin (64 µg/ml in LS 23), piperacillin/tazobactam (64 µg/ml in LS 47), and piperacillin (64 µg/ml in LS 58). Aztreonam, cefepime, ceftazidime, ceftriaxone, piperacillin/ tazobactam, piperacillin, and ticarcillin/L-clavulanate showed increased MIC values in treated LS 38. Piperacillin (in LS 23) and ticarcillin/K-clavulante (in LS 47) also showed slight increase in MIC values as compared to control (Table 2). Rest of antimicrobials did not show any change in MIC values after biofield treatment in clinical MDR isolates of P. aeruginosa.

	LS 22	-S 22 LS 2		LS 23		LS 38		LS 47		
Antimicrobial	С	т	с	т	С	т	с	т	С	т
Amikacin	>32	>32	≤ 16	≤ 16	>32	>32	>32	>32	>32	>32
Amoxicillin/K-clavulanate	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8
Ampicillin/sulbactam	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8	>16/8
Ampicillin	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Aztreonam	>16	16	>16	≤ 8	16	>16	>16	>16	>16	>16
Cefazolin	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Cefepime	>16	>16	>16	≤ 8	≤ 8	>16	>16	16	>16	>16
Cefotaxime	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32
Cefotetan	>32	>32	>32	≤ 16	>32	>32	>32	>32	>32	>32
Cefoxitin	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Ceftazidime	>16	>16	>16	≤ 8	≤ 8	>16	>16	>16	>16	>16
Ceftriaxone	>32	>32	>32	≤ 8	32	>32	>32	>32	>32	>32
Cefuroxime	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Cephalothin	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16
Chloramphenicol	>16	>16	>16	≤ 8	>16	>16	>16	>16	>16	>16
Ciprofloxacin	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
Gentamicin	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8
Imipenem	>8	>8	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	>8	8
Levofloxacin	>4	>4	>4	>4	>4	>4	>4	>4	>4	>4

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Meropenem	>8	>8	>8	≤ 4	≤ 4	≤ 4	≤ 4	≤ 4	>8	8
Nitrofurantoin	>64	>64	>64	64	>64	>64	>64	>64	>64	>64
Norfloxacin	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8
Piperacillin/tazobactam	>64	>64	≤ 16	≤ 16	≤ 16	>64	>64	64	64	64
Piperacillin	>64	>64	64	>64	≤ 16	>64	>64	>64	>64	64
Tetracycline	8	8	>8	>8	>8	>8	>8	>8	>8	>8
Ticarcillin/K-clavulanate	>64	>64	64	64	64	>64	64	>64	>64	>64
Tobramycin	>8	>8	>8	>8	>8	>8	>8	>8	>8	>8
Trimethoprim/sulfamethoxazole	>2/38	>2/38	>2/38	>2/38	>2/38	>2/38	>2/38	>2/38	>2/38	>2/38

Table 2: Minimum inhibitory concentration (MIC) of multidrug resistant lab isolates of Pseudomonas aeruginosa.

Increase in multidrug resistant and associated infections caused by P. aeruginosa has become a challenging for clinicians task to select anti-pseudomonal antimicrobials. Piperacillin/tazobactam, ceftazidime, aztreonam, imipenem, meropenem, amikacin, and ciprofloxacin are the best choice of antimicrobials against infections caused by P. aeruginosa. Apart from monotherapy, combination therapy, intravenous injections, and inhaled antibiotics are frequently preferred [30]. Biofield treatment has significantly decreased the MIC values in most of the tested antimicrobials such as piperacillin/ tazobactam, ceftazidime, aztreonam, imipenem, and meropenem. P. aeruginosa can exhibits resistance mechanisms, such as aminoglycoside-modifying enzymes, porin loss, efflux pumps, and various target site modifications [31]. Biofield treatment might act on enzymatic or genetic level which might affects the resistance mechanism, which may lead to alter the sensitivity pattern of most of the tested antimicrobials.

Biochemical and biotype number study

Biochemical study results and biotyping of control and biofield treated MDR lab isolates of P. aeruginosa are summarized in Table 3 and 4. Out of total tested 33 biochemicals, 16 were reported with altered biochemical reactions (48.48%) after biofield energy treatment MDR lab isolates of *P. aerugonisa*. Acetamide, arginine, in nitrofurantoin, malonate, and oxidase showed negative reaction (i.e. (+) positive to (-) negative) while positive reaction (*i.e.* (-) negative to (+) positive) was found in arabinose, glucose, hydrogen sulfide, oxidation-fermentation, galactosidase, rhamnose, sorbitol, and sucrose in LS 23 isolate. Esculin hydrolysis, glucose, lysine, raffinose, sorbitol and sucrose showed positive reaction, while acetamide and arginine showed negative reaction as compared to control in LS 38. Only change in acetamide was reported in LS 58 i.e. negative reaction as compared to control. Rest of biochemicals did not show any alteration in their reaction after biofield treatment. LS 22 and LS 47 did not show any change in biochemical reaction as compared to control. P. aeruginosa is a glucose non fermenter, motile, shows oxidase positive, glucose negative, and Voges-Proskauer negative reaction as a characteristic feature. Biochemical reactions of control MDR isolates of P. aeruginosa were well supported with literature data [32].

Based on the biochemical results, significant alteration in biotype numbers were observed in three biofield treated MDR isolates *i.e.* LS 23, LS 38, and LS 58 as compared to control. A very rare biotype number (7400 4263) was found in treated LS 38, without any special characteristics (*i.e.* green pigment) as compared to control (0206 3736). New organism was identified as *Citrobacter freundii* complex with changed biotype number 7302 0052 in LS 23 after biofield treatment on day 10 with respect to control (0206 3336) (Table 4).

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		LS	22	LS	23	LS	38	LS	47	LS	58
Code	Biochemical	С	т	с	т	С	т	с	т	с	т
ACE	Acetamide	+	+	+	-	+	-	-	-	+	-
ADO	Adonitol	-	-	-	-	-	-	-	-	-	-
ARA	Arabinose	-	-	-	+	-	-	-	-	-	-
ARG	Arginine	+	+	+	-	+	-	+	+	+	+
CET	Cetrimide	+	+	+	+	+	+	+	+	+	+
CF8	Cephalothin	+	+	+	+	+	+	+	+	+	+
CIT	Citrate	+	+	+	+	+	+	+	+	+	+
CL4	Colistin	-	-	-	-	-	-	-	-	-	-
ESC	Esculin hydrolysis	-	-	-	-	-	+	-	-	-	-
FD64	Nitrofurantoin	+	+	+	-	+	+	+	+	+	+
GLU	Glucose	-	-	-	+	-	+	-	-	-	-
H2S	Hydrogen sulfide	-	-	-	+	-	-	-	-	-	-
IND	Indole	-	-	-	-	-	-	-	-	-	-
INO	Inositol	-	-	-	-	-	-	-	-	-	-
K4	Kanamycin	+	+	+	+	+	+	+	+	+	+
LYS	Lysine	-	-	-	-	-	+	-	-	-	-
MAL	Malonate	+	+	+	-	+	+	-	-	+	+
MEL	Melibiose	-	-	-	-	-	-	-	-	-	-

NIT	Nitrate	+	+	+	+	+	+	-	-	+	+
OF/G	Oxidation-Fermentation	+	+	-	+	+	+	+	+	+	+
ONPG	Galactosidase	-	-	-	+	-	-	-	-	-	-
ORN	Ornithine	-	-	-	-	-	-	-	-	-	-
OXI	Oxidase	+	+	+	-	+	+	+	+	+	+
P4	Penicillin	+	+	+	+	+	+	+	+	+	+
RAF	Raffinose	-	-	-	-	-	+	-	-	-	-
RHA	Rhamnose	-	-	-	+	-	-	-	-	-	-
SOR	Sorbitol	-	-	-	+	-	+	-	-	-	-
SUC	Sucrose	-	-	-	+	-	+	-	-	-	-
TAR	Tartrate	-	-	-	-	-	-	-	-	-	-
TDA	Tryptophan Deaminase	-	-	-	-	-	-	-	-	-	-
TO4	Tobramycin	+	+	+	+	+	+	+	+	+	+
URE	Urea	-	-	-	-	-	-	-	-	-	-
VP	Voges-Proskauer	-	-	-	-	-	-	-	-	-	-
C: Contr	rol; T: Treatment; LS: Lab Is	olate	e; -: r	negat	tive;	+: po	ositive	э.			

Table 3: Effect of biofield treatment on *Pseudomonas aeruginosa* to the vital processes occurring in living organisms.

lsolate s	Group	Biotype Number	Organism Identification	Special characteristics
1.0.00	с	2063736	P. aeruginosa	Green pigment
LS 22	Т	2063736	P. aeruginosa	Green pigment
LS 23	С	2063336	P. aeruginosa	Green pigment
LS 23	Т	73020052	Citrobacter freundii	NA
LS 38	С	2063736	P. aeruginosa	Green pigment
LS 38	Т	74004263	P. aeruginosa	NA
10.47	С	2041732	P. aeruginosa	Green pigment
LS 47	Т	2041732	P. aeruginosa	Green pigment
10.50	С	2063736	P. aeruginosa	Green pigment
LS 58	т	2061736	P. aeruginosa	Green pigment

 Table 4: Effect of biofield treatment on Pseudomonas aeruginosa to distinguishing feature of the genotype.

Continuous development of resistance in pathogenic microbes against antimicrobial has been advanced and persistent. Alternative treatment strategies are adopted in case of infections but biofield treatment might be a great approach to alter the sensitivity pattern of antimicrobials against *P. aeruginosa*. As a part of energy medicine, biofield treatment is increasingly used in biomedical health care system. Mr. Trivedi's biofield treatment against pathogenic microbes were well established [15-17]. Biofield treatment might induce significant changes at genetic and/or enzymatic level, which may act on receptor protein of microorganism [33], so that most of tested antimicrobials might showed better susceptibility pattern, decreased MIC values, and altered biochemical reactions against treated *P. aeruginosa* as compared to respective control.

Conclusion

Our study showed the changing trend in antimicrobial sensitivity, MIC values, biochemical reactions, and biotype number after Mr. Trivedi's biofield treatment in clinical MDR lab isolates of *P. aeruginosa*. On the basis of changed biotype number in three isolates after biofield treatment, new organism was identified as *Citrobacter freundii* in LS 23 of biotype number 73020052. On the basis of improved sensitivity and decreased value of MIC in some of the currently resistant antimicrobials used against MDR *P. aeruginosa*, it is assumed that biofield treatment could be applied in biomedical health care system to improve the antimicrobial sensitivity pattern.

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Conflict of Interest

The authors declare that they have no competing interest.

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