

ORIGINAL ARTICLE

Relation between Drinking Water Contamination and Gastroenteritis

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ABSTRACT

Key words:

Bacteriological examination, Drinking water, Gastroenteritis

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Background: Water is essential for supporting public health and life without water is impossible. Microbial guidelines seek to ensure that drinking water is free of microorganisms that can cause disease. **Objectives:** This study focused on examination of drinking water (100 samples) from different community water supplies in Tanta city, Egypt for bacterial contamination and relationship between drinking water and gastrointestinal manifestations. **Methodology:** Drinking water samples were collected from different sources and places and subjected to three tests: a) measurement of physical characters of water pH, turbidity and free residual chlorine b) heterotrophic bacterial count technique c) multiple tube fermentation technique for identification of *E.Coli* and *Strept faecalis*. **Results:** Samples conformed to standard specifications bacteriologically and physically were 80% and 90% respectively. Samples with abnormal pH, turbidity and free residual chlorine were 2%, 8% and 14% of respectively. The incidence of total coliforms, *E.Coli* and *Strept faecalis* was 17%, 5% and 2% respectively. Attacks of GIT manifestations were present in people in 20% of places. Abnormal turbidity >1 NTU was significant with total coliforms. Total coliforms ≥ 2.2 , abnormal turbidity >1 NTU and heterotrophic bacterial count >500 at 37°C were significant with attacks of gastrointestinal manifestations. **Conclusion:** Ground water better than filtered water and less liable to microbial contamination. Turbidity is a good indicator for total coliforms and is significant with attacks of GIT manifestations. Use of water filters (7 stages) in all houses is recommended for improvement of water criteria.

INTRODUCTION

Water is essential for supporting public health, and providing food security. Contaminated tap water by gastrointestinal pathogens remains one of the most important causes of gastrointestinal diseases especially in children below 5 years¹.

It is estimated that about 3.4 billion people in developing countries are highly vulnerable to water insecurity which developed countries have overcome through massive technological and management investments².

The main reason of microbial contamination is due to the intermixing of sewer lines with drinking water supply lines, thus leading to microbial contamination and poor water quality³.

Waterborne diseases are those diseases that are transmitted through the direct drinking of water contaminated with pathogenic microorganisms and mostly are concentrated on children in developing countries⁴. Various types of bacteria/ viruses are found in inadequately treated water which at a level outside of identified limits, may reflect a problem in the treatment process or in the integrity of distribution system⁵.

So the aim of this study was to examine drinking water from different community water supplies in Tanta city, Egypt for bacterial contamination and study of relationship between drinking water and gastrointestinal manifestations.

METHODOLOGY

In this study, 100 samples of drinking water were collected from different sources and places in Tanta city, Egypt, within a period of six months (November 2016 to April 2017)

The samples were divided according to the followings:

Sources of water:

Filtered water (51samples): Raw water treated with coagulation, sedimentation, filtration and disinfected by chlorination and pumped to different places through pipes.

Ground water (39samples): Water that systems pump and treat from aquifers (natural reservoirs below the earth's surface) and also pumped to different places through pipes.

Bottled water (10samples): Water sold in the markets.

Places of water supply: *houses* (28samples), *educational institutes* (26samples) which included schools and nurseries, *health buildings* (12samples) which included hospitals and health centers, *outdoors* (12samples) included samples from different sporadic places (restaurants and tap water outside buildings), *bottled water* (10samples) water sold in the markets, *administration buildings* (7samples) which included Health Administration Building, and others and *mosques* (5samples).

Routes of drinking water handling: *tap water* (69samples) which included water from a piped supply which came from either filtered water or ground water, *filter water* (15samples) which included water came from filters, *bottled water* (10samples), *tank water* (3samples) from above houses or buildings, *jerkin water* (2samples) which was collected in jenkins and delivered to houses, *cooled water* in the street (1sample).

The following data were collected:

Information on household members, socio-economic status, method of drinking water handling, household sanitation and route of delivery of water, presence of repeated attacks of gastrointestinal manifestations including watery diarrhea, soft diarrhea, vomiting, nausea or abdominal cramps which may be due to consumption of drinking water in the last seven days before collecting the sample with exclusion of other causes of GIT manifestations e.g irritable bowel syndrome, crohn's disease, ulcerative colitis, celiac disease, or another condition with vomiting such as pregnancy.

An informed consent was obtained from all participants in this study. Ethical approval for this study was provided by Ethics and Research Committee, Faculty of Medicine, Tanta University.

The samples were subjected to the following procedures:

Sampling and processing:

Water sample (500ml) was collected from each site in a sterile glass bottle after flaming the tap opening, examined within 6 hours of collection or within 24 hours when retained in ice containers, 2.5cm space was allowed in the bottle for good mixing preparatory to examination. For bacteriological examination, in the laboratory we added 0.5ml of sodium thiosulfate (Na₂S₂O₃) broth (Hi Media, India) for neutralization of residual chlorine in water⁶.

Estimation of the physical characters of water:

Free residual chlorine test: by commercial visual comparator technique using Commercial visual comparator 705 (Orbeco Hellige, USA) .The accepted levels of free residual chlorine is from 0.2-5mg/liter⁷.

Measurement of turbidity: using turbidity meter (VELP, Scientifica, Italy). The accepted levels of turbidity must be below 1NTU⁷.

Measurement of pH: by electronic pH method using pH meter (Willis Tower Watson (WTW), Germany).The accepted level of drinking water pH from 6.5-8.5⁸.

Bacteriological examination of water⁷:

Identification of E.coli:

By multiple tube fermentation technique for estimation of the most probable number index(MPN) using lauryl tryptose broth, brilliant green lactose bile broth and tryptone water(oxid, England) (Table 1).

Identification of Streptococcus fecalis: by multiple tube fermentation technique for estimation of MPN using Azide dextrose broth, Bile aesculin agar (oxid, England), catalase test (negative) and gram staining (gram positive cocci) (Table 1).

Identification of heterotrophic bacteria: by heterotrophic plate count test using water plate count agar (oxid, England) at 22°C and 37°C.

Table 1: MPN index for various combinations of positive and negative results when five 10-ml tubes are used⁹.

Number of Tubes Giving Positive Reaction out of 5 of 10 mL each	MPN Index per 100 mL
0	<2.2
1	2.2
2	5.1
3	9.2
4	16
5	>16

MPN: Most probable number index

Statistical analysis:

Was done using SPSS(Version 20.0).Qualitative data were described using number and percent. Intergroup comparison was performed using Chi-square test, OR: Odds ratio used for finding the strength of association between two groups, CI: Confidence interval, LL: Lower limit, UL: Upper Limit which determine the accuracy level of the estimated mean we have calculated. *P*value< 0.05 was considered statistically significant.

RESULTS

Our study included 100 water samples: 51% filtered water, 39% from ground water and 10% bottled water. Most of samples were collected from houses 28% and 87% of places with good socio-economic status and house hold sanitation. The route of handling of drinking water was mostly from tap water(69%)and filter water 15%.It was reported that 80%,90% of samples were conformed to standard specifications bacteriologically and physically respectively(data not shown).

According to physical characters:

It was found that 98% of samples with normal pH (6.5-8.5) while 2% with pH < 6.5. It was found that 92% of samples with normal turbidity \leq 1NTU, while 8% of samples were >1 NTU. It was found that 86% of samples with normal free residual chlorine (0.2-5) mg/l while 14% were abnormal, 11 samples were below 0.2 mg/l while 3 samples were above 5 mg/l (Table 2).

It was found that good socio-economic status and house hold sanitation were statistically significant with normal turbidity ($p=0.009$) while the tap water as a route of handling of drinking water was statistically significant with abnormal turbidity ($p=0.003$) and normal free residual chlorine ($p<0.001$). Filtered water as a source of water was statistically significant with normal free residual chlorine ($p<0.001$) while bottled water was statistically significant with abnormal free residual chlorine ($p<0.001$) (Table 3).

Heterotrophic bacterial count technique:

It was found that 8% of samples had average log heterotrophic bacterial count > 2.6 cfu/ml at 22 °C, while 13% of samples had average log heterotrophic bacterial count >2.6 cfu/ml at 37 °C (Table 2).

According to bacteriological identification: it was found that the incidence of total coliforms, *E.Coli* and *Strept faecalis* was 17%, 5% and 2% respectively (Table 2). According to *E.Coli* incidence, 5 samples were positive 3 from filtered water and 2 from ground water, as regard route of water handling, all 5 positive samples were from tap water, however, bottled water and filter water were not contaminated by *E.Coli* (Table 3). The incidence of *Strept faecalis* among 100 collected samples was 2% of samples, one sample from filtered water and the other from ground water, according to route of water handling, there was one sample from tap water and the other from filter water (3 stages), however, bottled water was not contaminated by *Strept faecalis* (Table 3).

Table 2: Distribution of the studied samples according to physical, most probable number and heterotrophic bacterial count (n=100)

Physical parameters	No.	%
PH		
Normal	98	98.0
Abnormal	2	2.0
Turbidity		
Normal	92	92.0
Abnormal	8	8.0
Free residual chlorine		
Normal	86	86.0
Abnormal	14	14.0
MPN/100ml		
Total coliforms		
<2.2	83	83.0
\geq 2.2	17	17.0
E.coli		
<2.2	95	95.0
\geq 2.2	5	5.0
Strept faecalis		
<2.2	98	98.0
\geq 2.2	2	2.0
Log HBC/ml		
At 22°C		
\leq 2.6	92	92.0
>2.6	8	8.0
At 37°C		
\leq 2.6	87	87.0
>2.6	13	13.0

HBC: Heterotrophic bacterial count. **MPN:** Most probable number index

Normal values of **PH**(6.5-8.5), **turbidity** (\leq 1NTU) and **free residual chlorine**(0.2–5)mg/l¹⁰. Accepted values of **MPN/100L** of samples <2.2 for total coliforms, *E.coli* and *Streptococcus faecalis*, also log **heterotrophic bacterial count** \leq 2.6 cfu/ml at 22°C and 37°C⁷.

Table 3: Relation between physical, bacteriological characters of water and different variables (n=100)

Variables		Source of water			Socio-economic status and house hold sanitation		Route of handling of drinking water						
		Filtered water	Ground water	Bottled water	Good	Bad	Tap water	Filter water	Tank water	Cooled water	Jerkin water	Bottled water	
PH	Normal (n = 98)	No.	49	39	10	86	12	67	15	3	1	2	10
		%	50.0	39.8	10.2	87.8	12.2	68.4	15.3	3.1	1.0	2.0	10.2
	Abnormal (n = 2)	No.	2	0	0	1	1	2	0	0	0	0	0
		%	100.0	0.0	0.0	50.0	50.0	100.0	0.0	0.0	0.0	0.0	0.0
P		P = 0.597			P = 0.244		P = 1.000						
Turbidity	Normal (n=92)	No.	49	33	10	83	9	64	15	0	1	2	10
		%	53.3	35.9	10.9	90.2	9.8	69.6	16.3	0.0	1.1	2.2	10.9
	Abnormal (n=8)	No.	2	6	0	4	4	5	0	3	0	0	0
		%	25.0	75.0	0	50.0	50.0	62.5	0.0	37.5	0.0	0.0	0.0
P		P=0.111			P=0.009*		P=0.003*						
Free residual chlorine	Normal (n=86)	No.	51	35	0	75	11	67	15	2	1	1	0
		%	59.3	40.7	0.0	87.2	12.8	77.9	17.4	2.3	1.2	1.2	0.0
	Abnormal (n=14)	No.	0	4	10	12	2	2	0	1	0	1	10
		%	0.0	28.6	71.4	85.7	14.3	14.3	0.0	7.1	0.0	7.1	71.4
P		P < 0.001*			P=1.000		P<0.001*						
Total coliforms	Normal (n=83)	No.	42	31	10	74	9	58	12	1	1	1	10
		%	50.6	37.3	12.0	89.2	10.8	69.6	14.5	1.2	1.2	1.2	12.0
	Abnormal (n=17)	No.	9	8	0	13	4	11	3	2	0	1	0
		%	52.9	47.1	0.0	76.5	23.5	64.7	17.6	11.8	0.0	5.9	0.0
P		P=0.301			P=0.227		P=0.092						
E.Coli	Normal (n=95)	No.	48	37	10	83	12	64	15	3	1	2	10
		%	50.5	38.9	10.5	87.4	12.6	67.4	15.8	3.2	1.1	2.1	10.5
	Abnormal (n=5)	No.	3	2	0	1	1	5	0	0	0	0	0
		%	60.0	40.0	0	50.0	50.0	100.0	0	0	0	0	0
P		P=1.000			P=0.509		P=0.828						
Strept faecalis	Normal (n=98)	No.	50	38	10	86	12	68	14	3	1	2	10
		%	51.0	38.8	10.2	87.8	12.2	69.4	14.3	3.1	1.0	2.0	10.2
	Abnormal (n=2)	No.	1	1	0	1	1	1	1	0	0	0	0
		%	50.0	50.0	0.0	50.0	50.0	50.0	50.0	0.0	0.0	0.0	0.0
P		P=1.000			P=0.244		P=0.523						

χ^2 : Chi square test for comparing between the two categories. ^{FE}p: p value for Fisher Exact for Chi square test for comparing between the two categories, *Statistically significant at $p \leq 0.05$.

According to relation between physical and bacteriological characters, it was found that normal turbidity < 1NTU was significantly associated with normal count of total coliforms < 2.2 cfu/ml ($P=0.027$) (Table 4).

Table 4: Relation between Bacteriological and physical characters (n=100)

Physical parameters	Total coliforms				E. coli				Strept faecalis			
	<2.2 (n=83)		≥2.2 (n=17)		<2.2 (n=95)		≥2.2 (n=5)		<2.2 (n=98)		≥2.2 (n=2)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
PH												
Normal	81	97.6	17	100.0	93	97.9	5	100.0	96	98.0	2	100.0
Abnormal	2	2.4	0	0.0	2	2.1	0	0.0	2	2.0	0	0.0
P	1.000				1.000				1.000			
Turbidity												
Normal	79	95.2	13	76.5	88	92.6	4	80.0	90	91.8	2	100.0
Abnormal	4	4.8	4	23.5	7	7.4	1	20.0	8	8.2	0	0.0
P	0.027*				0.347				1.000			
Free residual chlorine												
Normal	70	84.3	16	94.1	81	85.3	5	100.0	84	85.7	2	100.0
Abnormal	13	15.7	1	5.9	14	14.7	0	0.0	14	14.3	0	0.0
P	0.453				1.000				1.000			

χ^2 : Chi square test for comparing between the two categories. ^{FE}p: p value for Fisher Exact for Chi square test for comparing between the two categories, *Statistically significant at $p \leq 0.05$.

The incidence of GIT manifestations among people was 20% of samples. We reported that average log heterotrophic bacterial count at 37 °C <2.6 cfu/ml, normal total coliforms count <2.2cfu/100ml and normal turbidity <1 NTU were indicators of good quality of

water as they were significantly associated with absence of gastrointestinal manifestations with P values(0.004,0.005 and 0.008)respectively and Odds ratios(6.64,5.26 and8.56) respectively (Table 5).

Table 5: Relation between attacks of gastrointestinal manifestations and socio-economic status, house hold sanitation, source of water, route of water handling and water quality variables

Variables	Attacks of gastrointestinal manifestations(n=100)				P	OR 95% C.I (L.L-U.L)
	Absence (n=80)		Present (n=20)			
	No.	%	No.	%		
Source of water					0.259	
Filtered water	51	63.8	10	50.0		1.76
Ground water	29	36.3	10	50.0		(0.65–4.72)
Socio-economic status and house hold sanitation					0.129	
Good	72	90.0	15	75.0		3.00
Bad	8	10.0	5	25.0		(0.86–10.45)
Route of handling of drinking water					0.114	
Tap water	55	68.8	14	70.0		1.06(0.36–3.08)
Filter water	12	15.0	3	15.0		1.0(0.25–3.94)
Bottled water	10	12.5	0	0.0		0.16(0.01–2.92)
Tank water	2	2.5	1	5.0		2.05(0.18–23.84)
Jerkin water	1	1.3	1	5.0		0.24(0.01–4.02)
Cooled water	0	0.0	1	5.0	12.38(0.49–315.8)	
Total coliforms					0.005*	
<2.2	71	88.8	12	60.0		5.26
≥2.2	9	11.3	8	40.0		(1.70–16.31)
E. coli					0.261	
<2.2	77	96.3	18	90.0		2.85
≥2.2	3	3.8	2	10.0		(0.44–18.34)
Strept faecalis					0.362	
<2.2	79	98.8	19	95.0		4.16
≥2.2	1	1.3	1	5.0		(0.25–69.53)
PH(6.5_8.5)					1.000	
Normal	78	97.5	20	100.0		0.77
Abnormal	2	2.5	0	0.0		(0.04–16.58)
Turbidity					0.008*	
Normal	77	96.3	15	75.0		8.56
Abnormal	3	3.8	5	25.0		(1.84–39.69)
Free residual chlorine					0.730	
Normal	68	85.0	18	90.0		0.63
Abnormal	12	15.0	2	10.0		(0.13–3.07)
Log HBC/ml					0.196	
At 22 °C						
<2.6	75	93.8	17	85.0		2.65
>2.6	5	6.3	3	15.0		(0.58–12.17)
At 37 °C						0.004*
<2.6	74	92.5	13	65.0	6.64	
>2.6	6	7.5	7	35.0	(1.92–22.94)	

HBC: Heterotrophic bacterial count **OR:** Odds ratio, **CI:** Confidence interval, **LL:** Lower limit, **UL:** Upper Limit.

DISCUSSION

Microbial hazards are said to represent greater threat than chemical hazards, and in developing countries account for 5.7% of the total global burden of water borne diseases¹¹. Globally, near 800 million people have no access to improved water sources and about 2.5 billion people do not have access to satisfactory sanitation, outbreaks of water borne diarrheal diseases still have a serious health threat worldwide¹².

This study focused on examination of drinking water (100 samples) from different community water supplies in Tanta city, Egypt for bacterial contamination and relationship between drinking water and gastrointestinal manifestations. Tanta city has two sources of drinking water: surface treated filtered water and ground water. The chief supply of drinking water to Tanta city is surface treated filtered water, while the chief supply of drinking water to villages of Tanta city is ground water.

pH value has an effect on the biological and chemical reactions, also controls the metal ion solubility and thus it affects the natural aquatic life and could control the pathogenic microorganism growth¹³. In the present study it was found that pH was normal in 98% of samples, free residual chlorine was normal in 86% of samples and turbidity in 92% of samples was normal (≤ 1 NTU). In accordance with our study, a Pakistani study done on 648 samples taken from 57 villages to test for microbial quality found that the average value of pH was 7.5, the amount of residual chlorine of samples was in the range of 0.12 and 0.53 mg/L, the average value of turbidity was 0.67 NTU, in 99.5% of samples turbidity was lower than 5 NTU¹⁴. However, an Egyptian study in Assiut Governorate noticed alkaline pH values in all sites. The alkalinity of water was caused mainly due to OH, CO₃, HCO₃ ions in which are formed by dissociation of inorganic compounds¹³. The variation in pH depends on the presence of the ions and their total conductivity and the temperature of water during measurement¹⁵.

Turbidity is the reflection of the total suspended matter and it interferes with proper disinfection¹⁶. In the present study it was found that turbidity in 92% of samples was normal (≤ 1 NTU), unlike an Egyptian study which found the average turbidity of river Nile samples at Assiut Governorate increased gradually during winter season (15 NTU recorded) this is due to the level of surface water in the study area is generally decreasing at this time¹³.

Good socio-economic status and tap water as a route of water handling were associated with normal turbidity. Also we noticed an association between abnormal free residual chlorine and bottled water, this was because chlorination isn't used as a method for

bottled water disinfection, while ozonization and copper-silver ionization are used. This is in agreement with an Indonesian study which showed that physical characters of water were affected by place of residence, island of residence and were significantly associated with the safety of domestic water supply because urban areas more likely used piped or improved water supply¹⁷.

Our study showed that total coliforms incidence among collected samples was 17%, the incidence of *E. coli* was 5% of samples and the incidence of *Streptococcus faecalis* was 2%. This was in agreement with an Egyptian study done in Zagazig City that found the incidence of total coliforms among 300 tap water samples was 12%, out of them 5.33% were *E. coli*¹⁸. However, our study incidence was less than the incidence detected by a Turkish study which reported that 30% of their tap water samples isolated from coastal region of Northern Turkey were contaminated by coliform bacteria¹⁹. These differences may be attributed to the larger study areas which included different environmental regions unlike our study which is restricted to a limited area of Tanta city.

We reported that the average log MPN of total coliforms and *E. coli* was 13.22 and 5.2/100 ml respectively. In accordance with our study a Pakistani research was done on 648 samples from 57 villages which found that the average log MPN of total coliforms and fecal coliforms were 15.54 and 6.06/100 ml respectively¹⁴.

We used bacterial plate count technique for isolation of heterotrophic bacteria at 22°C and 37°C and we found that the average log of total bacterial count at 22°C (92 samples were ≤ 2.6 cfu/ml and 8 samples were > 2.6 cfu/ml) and at 37°C (87 samples were ≤ 2.6 cfu/ml and 13 samples were > 2.6 cfu/ml). An Egyptian study measured the average log total bacterial count at different sites of Nile water which reached at 37°C 6.4 cfu/100 ml in El-Giza district, followed by Helwan, Shubbra-El-Khema and lastly Embaba being 5.8, 5.63 and 4.6 cfu/100 ml respectively. Also, the highest average log count at 22°C reached 6.2 cfu/100 ml in both Helwan and El-Giza regions, while Shubbra El-Khema and Embaba regions recorded 5.42 and 2.9 cfu/100 ml²⁰. Another study was done in Cairo segment which reported that the log total bacterial count of Nile water ranged from 4.1 to 7.4 cfu/100 ml at 22°C, while it reached from 4.1 to 7.3 cfu/100 ml at 37°C²¹. These differences may be due to different techniques, different number of samples and different geographical distribution.

As we reported 20%, 10% of samples were not conformed to standard specifications bacteriologically and physically respectively, these percentages were lower than those reported by an Indian study which reported that out of 1317 total samples, 565 samples

(42.9%) were found to be unsatisfactory²². The difference may be due to large number of samples, different geographical and environmental conditions.

The incidence of total coliforms and *E. coli* in surface treated filtered water was 52.9% and 60.0% from the total abnormal samples respectively that was higher than their incidence in ground water 47.1% and 40.0% respectively without significance, while there was no difference between the two sources in case of *Strept faecalis* with 50.0% incidence. This may be due to high prevalence of pollution, population density and more far from the main plant water station in areas supplied with surface treated filtered water²⁰. That is supported by another study done in Egypt in Zagazig city which reported that 68.75% of *E. coli* were isolated from areas with highest population density, only 18.75% of samples were isolated from areas of intermediate density and 12.5% of samples from lower population density. It was observed that all isolates were away from the main water plant in the western area of Zagazig¹⁸. On the other hand, an Indian study found a very highly deteriorated quality of ground water used for drinking purpose around different areas²³.

We reported that 20% of people in different places had attacks of gastrointestinal manifestations in the seven days preceding collection of the samples. This was higher than the incidence reported by an American study which reported a total of 99 (4.3%) individuals reported at least one symptom of GIT in the seven days preceding the survey²⁴. This difference may be due to environmental variations, individual changes and restrictions to standards of water quality.

In the present study we found that there was a significant associations between attacks of gastrointestinal manifestations and presence of total coliforms ≥ 2.2 , abnormal turbidity > 1 NTU and heterotrophic bacterial count > 2.6 cfu/ml at 37°C. However an American study found no association between GIT symptoms and turbidity²⁴. Another study in Urban Atlanta did not identify an association between treated water turbidity and GIT symptoms in emergency department visits²⁵. Also we reported that there was no significant association between source of water, socio-economic status, house hold sanitation and route of handling of drinking water and presence of attacks of gastroenteritis, however, other studies reported association between these factors and GIT symptoms^{24,26}. These differences may be attributed to lower number of samples in the present study and decreased awareness in some population sharing this study.

CONCLUSION & RECOMMENDATIONS

Periodic continuous monitoring of microbial quality of water is recommended to control the spread of pathogens transmitted by contaminated water.

Turbidity is a good indicator for total coliforms and predictor for possibility of attacks of GIT manifestations caused by water. Socio-economic status and route of water handling affect physical characters of water especially turbidity. Ground water is better than filtered water and less liable to microbial contamination. Use of water filters (7 stages) in all houses is recommended for improvement of water criteria.

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- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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