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Bacteriological Analysis of Sachet Water Sold in Some Municipalities Markets of Garoua Urbain City, Cameroon

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Abstract For some time now, there has been an increased proliferation of sachet packaged water sold in almost all sale points, even in the streets. With its doubtful quality, it becomes important to carry out a study on the bacteriological examination of those sachets of drinking water in the city of Garoua. 200 samples were collected for the bacteriological analysis for total coliforms, *Escherichia coli*, *Streptococci faecal*, and *Pseudomonas aeruginosa*. Results showed that 82% of the water sachets were contaminated. Total coliforms were isolated in 118 sachets, *Escherichia coli* in 74 sachets, *Streptococci faecal* in 48 sachets and *Pseudomonas aeruginosa* in 62 sachets of water. As associated risk factors, Hawkers sale type had a statistically significant relationship with contamination; handmade water bags are more likely to be contaminated. Moreover, long storage period favored the contamination of water sachets, the storage of the water bags in the coolers presented risks of contaminations and improper hygiene during the sale is a risk of contamination. Furthermore, during the analysis, one or more germs could be isolated. Accompanying measures by the public authorities to producers and vendors are necessary to improve the quality of these waters.

Keywords: *bacteriological quality, Escherichia coli, Streptococci faecal, total coliforms, Pseudomonas aeruginosa, sachet packaged water*

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1. Introduction

World health organization (WHO) has estimated that dirty water accounts for 9.1% of the diseases and 6% of the deaths recorded each year in the world [1]. Children are the first victims, since water is involved in 22% of diseases in children under fourteen. Diarrhea is the second leading cause of death in children under five and accounts for 525,000 child deaths per year [1]. Accessibility and availability of drinking water is an indicator of the level of development of a country [2]. Good quality drinking water is odorless, colorless, tasteless and free of faecal pollution, thus avoiding 80% of the diseases present in the environment [3]. Poor quality water is often responsible of intestinal infections, dysentery, hepatitis, typhoid fever, cholera and other possible pathologies [4]. However, an estimated 1.2 billion people do not have access to safe water [5]. In Cameroon, despite natural predispositions that allow it to have significant water resource, estimated at 18500 m^3 / year, the rate of access to the resource is only 32% [6]. The current level of water supply in Cameroon is yet to meet the current water needs of households and industries [6]. In the Northern region of Cameroon, only 24.7% of the population gets water from improved sources [6]. Thus, to participate in the improvement of water supply, local entrepreneurs have started producing water packaged in plastic bags. These waters are very popular because of their reduced cost for low income residents, their user-friendliness and especially because of their refreshing character given the very high temperatures in this region. Unfortunately, those waters are not subjected to the same quality constraints as

those of the major drinking water production companies. Furthermore, the conditions in which these waters are produced in sachets, their doubtful origin and their improper handling remain questionable. Thus, this study is aimed at assessing the microbiological examination of sachet packaged water sold in the city of Garoua and to highlight the risk factors associated with their contamination.

2. Material and Methods

2.1. Framework and Study Area

The study was conducted in the city of Garoua, capital of the North region of Cameroon. It is located in Bénoué basin at 9°18' N latitude and 13°24' E longitude, large sedimentary basin stepped with an average altitude between 200 and 250 m. The climate, of tropical type with Sudanese variant, is hot and dry, with 1000 mm of precipitation per year [7]. In Garoua, the heat can be torrid at the end of the dry season despite the shade provided by trees along the main streets. The average monthly temperature increases from 26°C in August to 40°C in March (extreme temperatures: 17°C and 40°C) with maxima in April and October, beginning and end of rains, and minima in December and August, marking the full dry season and full rainy season, respectively [8].

Samples were collected in the neighborhoods of the three municipalities of Garoua shown in Figure 1.

The questionnaires were filled in by the vendors. For those who did not know how to read and write, the questions were translated into their local languages and answers were filled out by interviewers while they were answering, without influencing their response.

2.2. Type and Period of Study

This longitudinal study was conducted in the capital city of the Northern region of Cameroon, between May and October 2017, to assess the bacteriological quality of water sachets sold in every corner of the city.

2.3. Sampling and Analyzes

The study was conducted in three stages:

- A phase of identification of the different brands of sachets sold in three municipalities of Garoua. Thus, three types of production were identified namely: industrial, semi-industrial and local.
- A phase of collection of bags of various brands found in the markets in the three municipalities of the city of Garoua. A total of 200 sachets of water of 500 ml each, were purchased from various points of sale (markets, bus stations, roadside shops and street vendors or Hawkers) of the city. The water sachets were kept in a cooler containing ice pack (4°C), transported away from light and analyzed within 24 hours. During the collection of water sachets, various activities were carried on the production and sale of packaged water in sachets (type of production, type of sale, method of supply, place of storage, storage time, hygienic practices) to identify contamination risk factors of water in sachets.

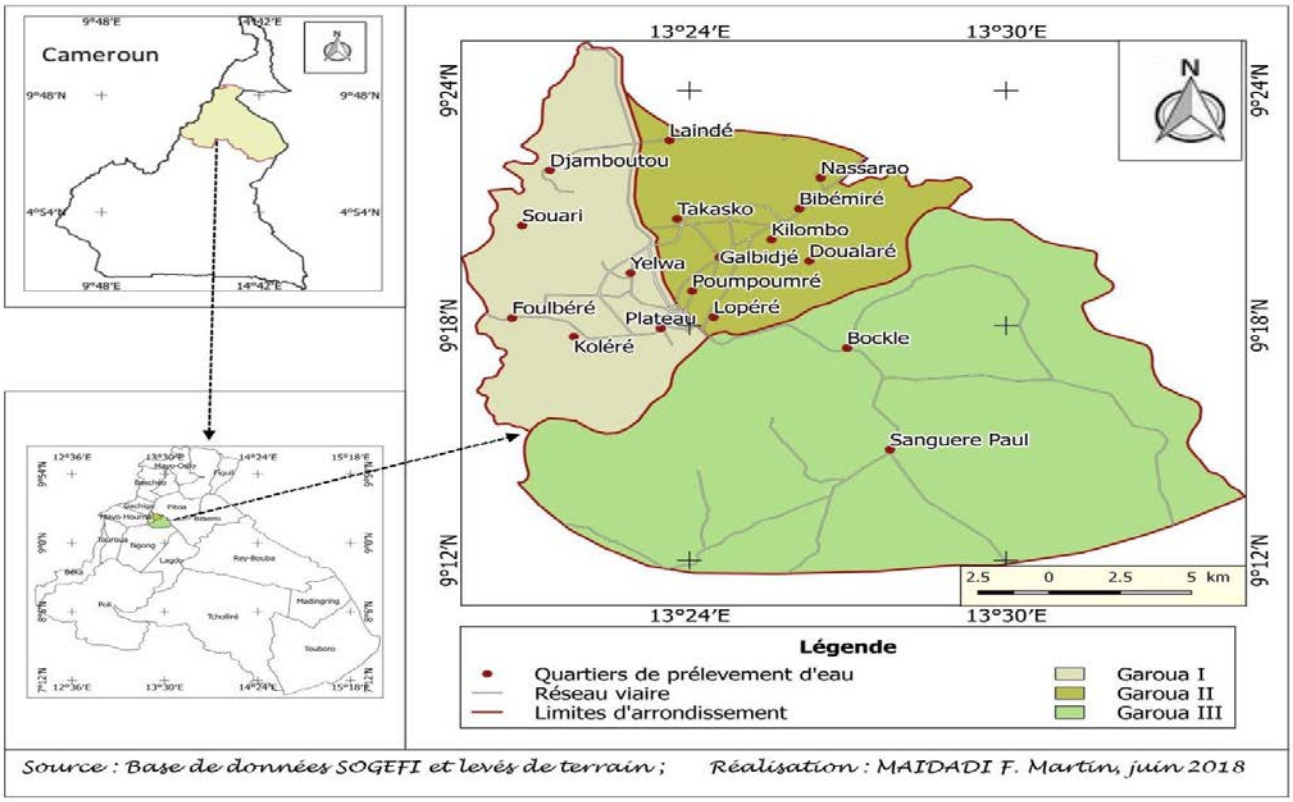

Figure 1. Map of Garoua region showing the sampling sites

The parameters below were categorized:

• Method of production of water in sachets: Industrial production, semi industrial and local;

• Types of sale: Hawkers and fixed sellers;

• How the vendors are supplied: Buying from the factory, or self-production;

• Methods of conservation: open air, refrigerator, cooler, buckets;

• Period of storage before the sale: less than 3 days, and more than 3 days;

• Hygienic practice: use of soap or not, for washing hand before serving sachet water

Some producers use public water supply while others use borehole water.

➢ A phase of detailed bacteriological analysis of water unit which was done in the water analysis laboratory of the Garoua Annex Pasteur Center of Cameroon. The membrane filtration method was used for the enumeration of bacteria present at very low concentrations in water [9]. In fact, 100 ml of water was filtered through a 0.45 µm pore diameter filter. The filter which retained the bacteria contained in the water was then deposited on a suitable culture medium. The followings were investigated:

- Indicators of faecal contamination: if the following organisms were observed alone or in combination: total coliforms, *Escherichia coli*, *Streptococci faecal*; all these germs were faecal germs, which signified a contamination of water by stool.

- *Pseudomonas aeruginosa*, its isolation in water was a sign of contamination by pollution, because it is a germ of the environment and is found everywhere.

- There were faecal and environmental co-contamination of the same bag. This implied an association between one or more of the faecal contamination indicators and *Pseudomonas aeruginosa.*

The isolation of total coliforms and *Escherichia coli* was done according to international standards [10]. For *Streptococci faecal*, the isolation was done on Slanetz medium and confirmed after 4 hours of incubation on BEA (Bile-Esculin-Azide) medium [11]. For the isolation of *Pseudomonas aeruginosa*, Cetrimide Agar and Nalidixic Acid were used [12]. The results were expressed as numbers of colony-forming units per 100 ml of water. Good quality water was defined as the absence of contamination indicators in 100 mL of water analyzed. The data were subjected to Microsoft Excel 2010 and analyzed using SPSS Statistics 22 software. P-value <0.05 was considered as statistically significant.

3. Results

200 samples of water bags were collected from the three municipalities of Garoua. 82 bags of water were collected in the municipality of Garoua I, 76 in the municipality of Garoua II and 42 in the municipality of Garoua III.

I. SOCIO-DEMOGRAPHIC CHARACTERISTICS

1. Characteristics of the vendors

The bags of water were taken from Hawkers and fixed vendors. Their hygienic practices were assessed during the sale period using a questionnaire asking if they were using the following means: use of soap, use of plain water for washing hands and utensils or no hygiene (Table 1).

Table 1. Frequency of the different characteristics of the vendors

Types of vendors	Hawkers	86	43
	Fixed	114	57
	Total	200	100%
Hygienic methods (washing of hands and utensils)	Soap	150	75
	Plain water	26	13
	Assume clean	24	12
	Total	200	100%

2. Characteristics of water bags

The different characteristics of the water bags are summarized in Table 2.

Table 2. Frequency of the characteristics of the sachets of waters

		Numbers	Percentage
Methods of supply	Buying from factory	140	70
	Self-production	60	30
	Total	**200**	**100%**
Storage site	Refrigerator	100	50
	Bucket	30	15
	Cooler	30	15
	Free air	40	20
	Total	**200**	**100%**
Types of production	Industrial	120	60
	Semi-industrial	30	15
	Home-made	50	25
	Total	**200**	**100%**
Stockage time	Between 1 and 3 days	138	69,0
	More than 3 days	62	31,0
	Total	**200**	**100%**

II. BACTERIOLOGICAL STUDY OF WATER BAGS

Figure 2 shows the proportion of water sachets according to their quality (uncontaminated or contaminated water) observed in bacteriological analysis.

A total of 200 sachets of water were collected at the various sale points and analyzed. Results revealed that 82% of the bags were contaminated and 18% uncontaminated.

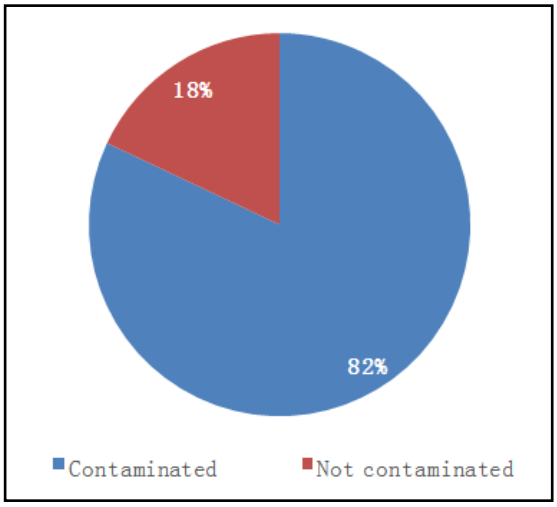

Figure 2. Proportion of water bags according to their quality

Figure 3 describes the proportions of the different contaminating germs identified in the water sachets. It can be seen that total coliforms accounted for 59% of germs found, followed by *Escherichia coli* (37%), *Pseudomonas aeruginosa* (31%) and *Streptococci faecal* (24%).

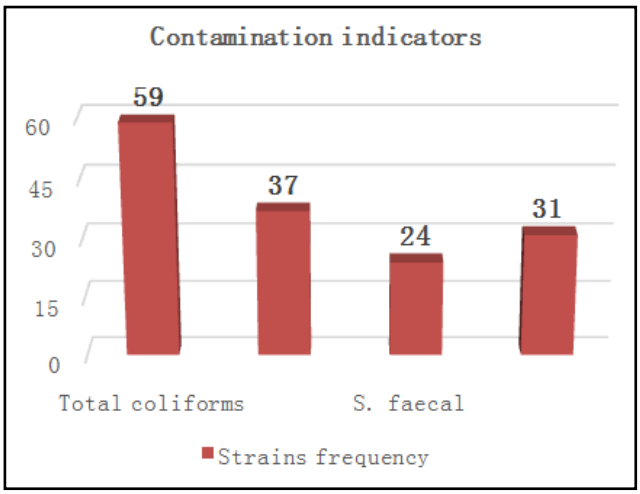

Figure 3. Distribution of isolated organisms in the analyzed water bags

The frequencies and co-existence of these germs are summarized in Table 3. More than half of the bags of contaminated water i.e 53.66%, revealed the presence of several indicators.

The different combinations of germs in the same sachet i.e between contamination indicators (total coliforms, *Escherichia coli* and *Streptococci faecal*) on one hand and between environmental germs and the faecal contamination indicators on the other hand explained the faecal and environmental origins of the contamination of Table 4.

Table 4 shows that environmental contaminations are highest, with a frequency of 43.88% followed by faecal contamination, 34.14%.

Table 5 presents the risk factors associated to bacterial contamination. In the univariate analysis, there was a statistically significant relationship between the following variables and bacterial contamination using Pearson's chi-square. These are: type of sale (p = 0.004), type of production (p<0.0001), place of storage of water bags (p<0.0001), storage period (p<0.0001) and the methods of hygiene used with p = 0.042. However, there was no statistically significant relationship with the method of supply (p = 0.173). Also, there was no interaction between the different variables influencing the bacteriological quality of bagged water in multivariate analysis.

Table 3. Distribution of indicators according to the type of contamination in the city of Garoua

Type of contamination	Indicators	Numbers	Frequency (%)	
Mono-contaminations N1 = 76	Total Coliforms	44	26.82	**46.34**
	Escherichia coli	10	6.10	
	Streptococci faecal	12	7.31	
	Pseudomonas aeruginosa	10	6.10	
Poly-contaminations N2= 88	Total Coliforms and *Escherichia coli*	18	10.97	**53,66**
	Escherichia coli and *Streptococci faecal*	08	4.8	
	Total coliforms and *Pseudomonas aeruginosa*	18	10.97	
	Total coliforms and *Streptococci faecal*	02	1.21	
	Total coliforms, *Escherichia coli* and *Streptococci faecal*	08	4.8	
	Total coliforms, *Escherichia coli* and *Pseudomonas aeruginosa*	16	9.75	
	Total coliforms, *Escherichia coli*, *Streptococci faecal*, and *Pseudomonas aeruginosa*	12	7.31	
	Others	06	3.66	
N= 164	Total	164	100%	100%

Table 4. Distribution of contaminated water bags according to the origin of the contamination in Garoua

Origins of contaminations	Numbers	Frequency %
Faecal contamination	56	34.14
Environnemental contamination	72	43.88
Co-contamination	28	17.10
None classified others	08	04.88
Total	164	100

Table 5. Risk factors of bacterial contamination of water sachets

Variables N= 200		Numbers	Contaminated sachet	Frequency of contaminated sachet %	OR	Univariate analysis P-value	Multivariate analysis p-value
Type of vendor	Hawkers	87	79	90.80	3.2 (1.4-7.5)	P=0.004	0.809
	Fixed	113	85	75.22			
Methods of supply	Buying from company	141	111	78.72		P=0.062	–
	Self-production	59	53	89.83			
Types of production	Industrial	120	102	85.0		p<0.0001	0.082
	Semi-industrial	30	16	53.3			
	Home-made	50	46	92.0			
Storage period	Between 1 and 3 days	139	127	91.36	6.8 (3.1-15.0)	p<0.0001	0.73
	More than 3 days	61	37	60.65			
Site of Storage	Refrigerator	101	89	88.11		p<0.0001	0.156
	Bucket	30	28	93.33			
	Icebox	30	28	93.33			
	Free air	39	19	48.71			
Hygienic methods	Use soap	151	125	82.78		P=0.042	0.533
	Use water	23	15	65.21			
	No hygiene	26	24	92.30			

4. Discussion

82% of the water bags collected from the city of Garoua showed signs of contamination either by faeces or by the environment. This high level of contamination highlights the problem of the quality of these waters mostly consumed in the city and indicates a real public health problem with regard to the risks incurred by the population. This same results were observed in Abidjan, where Aboli found that 79.4% of the water sold in plastic sachets, be it semi-industrial or hand-made, was unfit for consumption [13]. These levels of contamination are also reported by Edema in Nigeria with a proportion of 87% of contaminated bags [14].

Most of the indicators of water contamination were found alone in the sachet (total coliforms, *Escherichia coli*, *Streptococci faecal*) or sometimes in combination with at a level of 34.14%, which indicates a high faecal contamination of these substances. Apart from these indicators, *Pseudomonas aeruginosa* was also isolated singly or in association with other indicators (43.88%) of faecal contaminations which is an indication of contamination when handling or selling. This same finding has been reported by other authors. These authors also highlighted these indicators of contamination and *Pseudomonas aeruginosa* in sachet waters [15].

In the present study, 50% of total coliforms were isolated. This is higher than what is usually reported. However, it is known that total coliforms are not specific to the gut of humans or other warm-blooded mammals, but can also be found in the environment [16]. They are sensitive to chlorine, so their presence in water samples may indicate the existence of a biofilm or a lack of treatment efficacy as described in the literature [16].

The detection of 37% of bacteria, *Escherichia coli* is indisputable evidence of recent faecal contamination and indicates the potential presence of enteric pathogens [16]. This is a sign that, the water collected from the packaged sachets is most often already contaminated with faeces.

The 24% *Streptococci faecal* are generally excreted in the faeces of humans and other warm-blooded animals. They tend to survive longer in aqueous environments than *Escherichia coli*, they are more resistant to desiccation and chlorination. These are indicators of faecal pathogens, able to survive longer than *Escherichia coli* and complement the *Escherichia coli* tests. In general, they are sought to determine water quality when repairs have been made to distribution networks or where new pipes have been installed [17].

21% of *Pseudomonas aeruginosa* was found in the analyzed waters, which is similar to the percentage reported by Amna in Pakistan where they found a rate of 24% [18]. Known as the most common organism in the environment, it can be found in faeces, soil, water and sewers. It can multiply in aquatic environments and also on the surface of some organic materials in contact with water [17].Their presence in drinking water, especially in treated water is due to the fact that it is able to survive longer in the nutrient-poor environment by lowering its metabolism and resisting the disinfectant often used for water treatment [19].

10.97% association of total coliforms and *Escherichia coli*, 4.8% of *Streptococci faecal*, and *Escherichia coli* association, 4.8% association of total coliform, *Streptococci faecal, and Escherichia coli*, and 1.21 % of association between total coliforms and *Streptococci faecal* were detected. An association of total coliforms and *Streptococci faecal* was previously reported found in 10 sachets in Abidjan by Anne [20]. These associations are an incontestable proof of faecal origin of the contamination of these sachets. The association of these faecal germs with *Pseudomonas aeruginosa* in some sachets reinforces the fact that the environment contributes to the contamination of these waters.

In analyzing the risk factors, the contamination could be related to:

- The type of sale: Bagged waters are distributed in two different ways in the city of Garoua by Hawkers and fixed vendors. It was found that water bags collected from Hawkers were 3 times more contaminated than those collected from fixed vendors. This can be explained by the unhygienic practices of vendors. In fact, they do not virtually wash their hands before serving the bags to consumers. Indeed, hands are contaminated during greetings, by shaking hands according to the customary practices of the region, and also during defecation. The high level of faecal germs observed in this group of people has also been observed in Sierra Leone, where *Escherichia coli* and total coliforms bacteria were isolated on the outside of the water bags collected from Hawkers [21]. Furthermore, it has been found that street vendors use containers that sometimes do not have a lid, hence the risk of contamination by germs in the environment [22], highlighting the high prevalence of *Pseudomonas aeruginosa* in the present study.

Studies have reported that there is an increase in the rate of germs as the bags are transported from the place of production to the points of sale [22]. Dada from Nigeria also showed that the further the product moves away from the production site, the more the bacteriological quality deteriorates throughout the production chain [23]. This trend is related to the handling of sachets by individuals who are unaware of hygiene.

- The types of production

The quality of the water in sachets is influenced by the way they are produced. In fact, it was observed that the water bags produced in the local way were the most contaminated. This same observation was reported in Côte d'Ivoire [13], where they found a significant difference in contamination between hand-made and semi-industrial water sachets. This could be explained by the fact that these bags of water are bagged locally and without any respect of the hygienic conditions. As other authors have noted, the origins of these waters might be dubious [13] and would come from various uncontrolled sources such as, well water and boreholes that are 100% the most often contaminated [24]. In addition, it is believed that water gets contaminated at the time of making the bags, the filling of water and also upon closing the bags.

It was found that 85% of the water packaged in sachets produced industrially are contaminated. This same observation has been observed by other authors [21,22]. These contaminations may be due to:

- Unhygienic practices when bagging water at the plant, where some authors have noticed that workers do not wear masks or gloves, and otherwise do chats without a mask [22].

- The poor maintenance of the filtration system, where other studies have shown that, especially in the case of the charcoal filter, is at the origin of the bacterial growth [25].
- Other producers use ozone for water sterilization. However, the success of this process depends on the maintenance of the ozonator and the knowledge of the pH of the water. The ozonator must be replaced after 3 to 4 years of use. Ozone works best when the pH of the water is high and for a pH between 7 < pH <10, the half-life of ozone is 15 minutes [26]. However, in the case of sachet waters of the city of Garoua, their pH is acid [24]. Water acidity reduces the effectiveness of ozone if not added in large quantities. It is therefore necessary to master the pH of the water in order to adjust the necessary amount of ozone at the production plants [26].
- Finally, the storage of water bags in factories is done in insalubrious places and can be a source of contamination in industrial bagged water [22].

- The storage time

It was observed that when the storage period of these bags of water is greater than 3 days, the risk of contamination is multiplied by 3 (p = 0.0001, OR = 2.877); this same finding has been noted by other authors [27]. This is related to the handling of bags by individuals who are unaware of hygiene as reported in other circumstances. In fact, the longer the storage period, the more exposure to environmental indicators increase. Likewise, the higher the number of times the sachets are handled with the hands (when they are stored, or when the sachets are kept before, during or after the sale), the higher the rate of contaminated sachets.

5. Conclusion

At the end of this study, it should be noted that 82% of the water sachets sold to the population of the city of Garoua are unfit for human consumption. As a result, they constitute a heath concern for consumers. The non-adherence to the quality system of water production by both industrial and local producers, the unhygienic handling of the bags, during the sale and finally, the lack of hygienic practices by the vendors have led to the contamination of the consumers. The behavior of the staff, the quality of the plastic, the dilapidated water treatment system and the storage of the bags in dirty places before transport constitute risk factors for water contamination at the production plants. This study highlights the need for the establishment of a regulatory authority, authorizing the production of water sachets, the sale and quality control of water bags produced, distributed and sold in the market.

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All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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