



Research Paper

Investigation on children's handwashing behaviour and contamination levels in rural Cambodia

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ABSTRACT

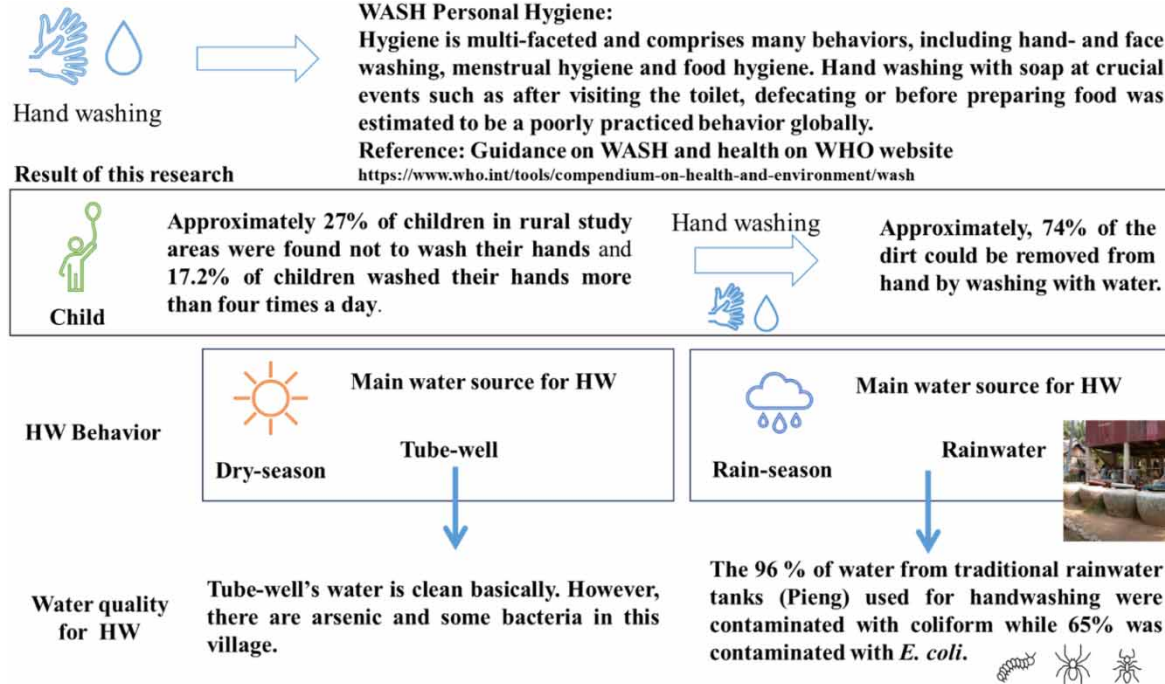
The present study aimed to investigate children's handwashing behaviour, contamination levels and qualities of water sources used for handwashing in rural Cambodia. A field survey was conducted by simple random sampling in Kandal Leu village of Kandal Province in 2014. Only 23 children were interviewed with the informed consent of their guardians, limiting our data analysis as descriptive. Concurrently, an adenosine triphosphate test kit was used to assess their hands' contamination levels. Their water sources for handwashing were also tested for *Escherichia coli* and coliform. As a result, approximately 27% of children in the study areas were found not to wash their hands and 17.2% of children washed their hands more than four times a day before and after eating, after touching dirt and after toileting. Approximately 74% of the dirt could be removed from hands by washing with water. Concurrently, approximately 96% of water from traditional rainwater jars (Pieng) used for handwashing was contaminated with coliform, while 65% was contaminated with *E. coli*. Cambodian children in our study area used polluted rainwater for handwashing in the rainy season. This study suggests that appropriate handwashing requires safe water and educational programs on environmental and personal hygiene for parents and children.

Key words: children, diarrhoea, handwashing, rural Cambodia

HIGHLIGHTS

- 27% of children under the age of 15 years in the study area were found not to wash their hands.
- 17.2% of children under the age of 15 years washed their hands more than four times a day in the study area.
- In the rainy season, children under the age of 15 years washed their hands using rainwater.
- Rainwater used for washing hands was polluted by coliform and *E. coli*.
- 74% of the dirt could be removed from hands by washing with water.

GRAPHICAL ABSTRACT



INTRODUCTION

Contaminated water and poor sanitation are among the leading causes of death in children under the age of 5 years (UNICEF 2023a). Without adequate water, sanitation and hygiene (WASH), children are at a high risk of contracting preventable diseases. In addition, diarrhoea is a leading cause of death in children and accounted for about 9% of all deaths among children under age 5 worldwide, in 2019. Approximately 1,300 young children die each day due to diarrhea, which is about 484,000 children a year (UNICEF 2022). The Kingdom of Cambodia's population was around 16 million, of which approximately 4.5 million (28.2%) were children under the age of 15 (NIS 2021). According to the World Bank Report, the infant mortality rate of Cambodia was 21 per 1,000 live births in 2021, which was relatively high among ASEAN countries, and ranked Cambodia the third worst, followed by Lao as the second, and Myanmar as the first (World Bank 2021). The leading causes of children's deaths is infectious diseases including diarrhoea, pneumonia and malaria (UNICEF 2023b). Interventions to prevent diseases, appropriate vaccines, safe drinking water and effective hand hygiene can reduce disease risks. Diarrhoea in rural Cambodia was closely related to the form of water used. Acute diarrhoea daily occurred in rural areas because of drinking surface water without treatment (UNICEF Cambodia 2019). Following the adoption of the global goals in late 2015, the Royal Government of Cambodia assigned the Ministry of Planning as the focal point for taking forward the development of the future Cambodian Sustainable Development Goals (CSDGs) framework (RGC 2018). However, the level of adaption and innovation was low at the indicator level. For instance, only five targets and six indicators have been adopted in the CSDGs framework for SDG 6, while it is eight targets and 11 indicators in the global framework (RGC 2018). The Cambodian government has planned sustainable access to an improved water supply for 100% of the rural population by 2025 (MRD 2011). The percentages of urban and rural population having access to safely managed drinking water service in their homes are 55 and 16%, respectively (MRD 2019). The percentages of urban and rural residents having access to basic water services are 95 and 70%, respectively (MRD 2019). Approximately 58.7% of rural households have access to an improved water supply, while 79% of the total households reported always treat their water for drinking (MRD 2019). Although many Cambodian people lived along watersheds, shallow groundwater was still the main source of drinking water due to the lack of safe water supplies and water treatment systems (Phan *et al.* 2010). Shallow groundwater, which is used as a drinking water source in rural Cambodia, could be contaminated with bacteria from toilets (Manago 2013). Likewise, a recent study revealed that both total coliform and *Escherichia coli* are found in tube wells, dug wells, ponds, canals and lakes, while the Cambodian Drinking Water

Quality Standard for total coliform and *E. coli* is not detectable per 100 mL (Sao *et al.* 2023). In addition, a recent study revealed that some inhabitants residing in the coastal area of Cambodia were exposed to contaminants of health concern (As, F⁻, NO₃⁻) (Phan *et al.* 2023), and experienced water salinization (Phan *et al.* 2021) and aesthetic properties through their water consumption (Phan *et al.* 2019). Drinking properly treated water is an effective measure against diarrheal disease, and at the same time, it is important to wash hands frequently with clean water (UNICEF Cambodia 2019). Several studies have been conducted on child hygiene and handwashing in Cambodia. One of them concerns the sanitation of primary school children. En & Gan (2011) surveyed the water sources and sanitation facilities of 40 primary schools and revealed that there was a correlation between educational level, gender and sanitary environment. Furthermore, this study suggested that hygiene education for uneducated parents was very important as well as for children. Nuki & Kitawaki (2016) investigated the economic situation and selection of handwashing agents in rural Cambodia by comparing the rate of reduction in handwashing contamination by using liquid soaps, bar soaps, dishwashing detergents, etc. It was found that the decontamination rate on hands was reduced by liquid and solid soaps to about 80%, while tableware and laundry detergents showed a degree of decontamination of about 60% (Nuki & Kitawaki 2016). Concurrently, it was found that the decontamination rate of handwashing with soap was approximately six times higher than that of handwashing with water, and households who could afford soaps for such handwashing were relatively wealthy (Nuki & Kitawaki 2016). A previous study investigated household practices related to disease transmission between animals and humans in rural Cambodia, and found that there was a correlation between washing hands with soap after handling live animals, and people with knowledge of zoonotic diseases had a habit of washing their hands before and after cooking (Osbjør *et al.* 2015). Likewise, Vong *et al.* (2009) showed that inadequate handwashing and contact with culled birds were risk factors for H5N1 virus infection. It needs collective efforts to get children to take these preventive actions daily. However, due to the effects of the COVID-19 pandemic, handwashing behaviour has changed significantly, and it is thought that a similar behavioural change has occurred in rural Cambodia too. This change in handwashing behaviour may have a lower mortality rate in children under the age of 5 years in the future. Therefore, the present study was to investigate children's handwashing behaviour and contamination level in rural Cambodia before the COVID-19 pandemic. This survey showed which water sources children used to wash their hands, the contamination level before/after washing and microbial qualities (coliform and *E. coli*) of water used for washing hands by rural Cambodian children.

MATERIALS AND METHODS

Study area

Kandal Leu village in Kean Svay District, Kandal Province located in the Mekong River basin is selected as the study area (Figure 1) because there is no intervention by international organizations – it avoids bias in the interview survey – no water pipes, and they use rainwater tanks as in traditional lifestyle areas. This Kandal Leu village is located about 40 km east of the capital Phnom Penh and has a population of about 900. An elementary school, a junior high school and a temple are built in the center of the surveyed village, and it is possible to access the Mekong River on foot to fetch water.

Field surveys

The field survey was conducted by a simple random sampling technique. The respondents voluntarily participated in the present study. We interviewed children under the age of 15 years ($n = 23$) about their handwashing behaviour, analysed the degree of contamination of their hands and measured the water quality of the water source for handwashing, after informed consent was obtained from their guardians in 2014. In addition, we applied qualitative inquiry to observe child participants' handwashing behaviour before and after eating, after touching soil and after toileting. Moreover, information on water practice and handwashing behaviour was collected at the Institute of Technology of Cambodia, the Royal University of Phnom Penh, the JICA Cambodia Office, and the Cambodian Bureau of Statistics.

The measurement of the contamination level on the hands of children in the study village

The contamination of hands was determined by a wiping test, using an adenosine triphosphate (ATP) test kit manufactured by Kikkoman Biochemifa Co., Ltd, Japan (Kikkoman Corporation 2023). The ATP wipe test not only measures bacteria and viruses, but also can quantitatively measure dirt and stains on fingers and hands. This method has been used in food manufacturing and services (Tanaka *et al.* 2020; Ching *et al.* 2021), hotels, cleaning and environmental hygiene fields (Aycicek

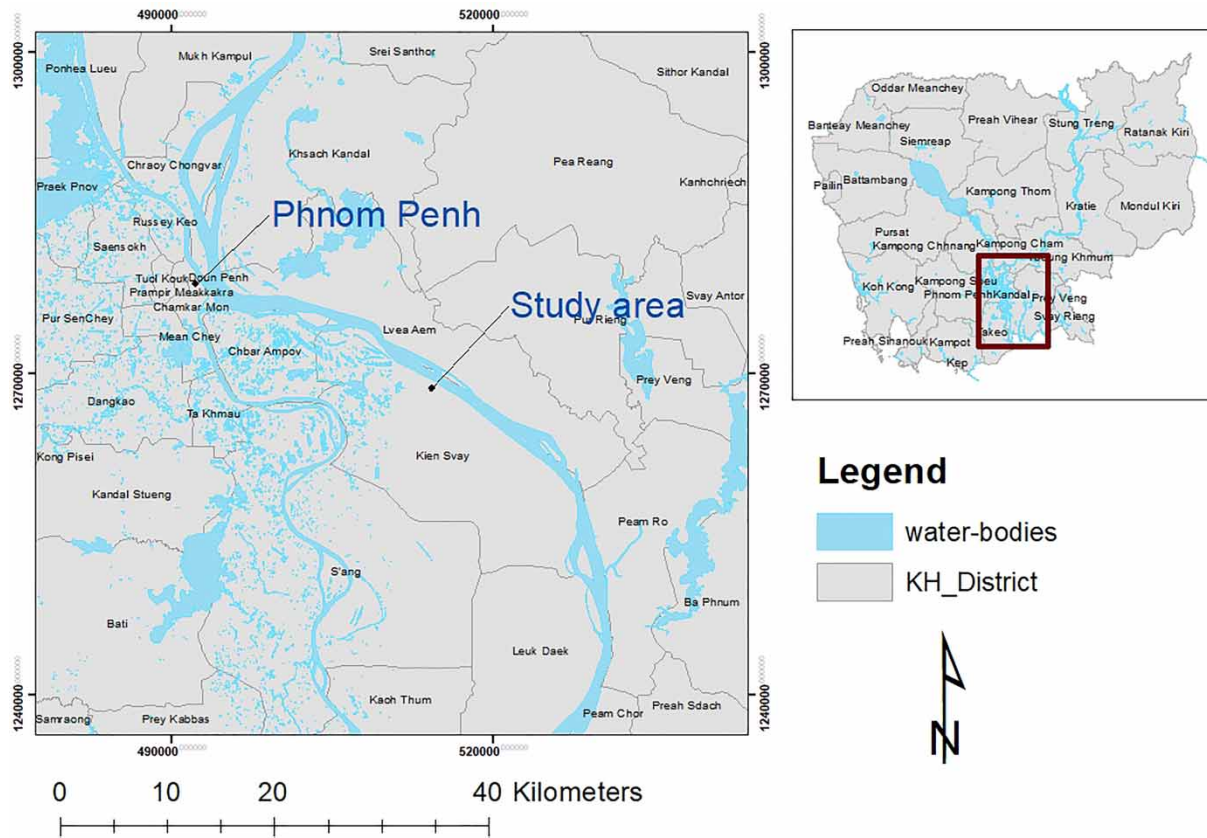


Figure 1 | Map of the study area, Kandal Leu Village.

et al. 2006; Omidbakhsh *et al.* 2014; Yi *et al.* 2020). This ATP test has also been used in the medical field (Bakke *et al.* 2019; Oie *et al.* 2020). The ATP wiping test is a technology that applies an enzymatic reaction to generate light by reacting with luciferase. The amount of ATP is proportional to the amount of luminescence. The amount of ATP is quantitatively analysed by measuring the amount of this luminescence with a measuring instrument. The kit was used to measure with approximately 5–10 wipes on the palms of children under the age of 15 years and was compared with the recommended value (Relative Light Unit – RLU) in Table 1.

The measurement of microbial contamination in water sources for handwashing

3-M Petrifilm *E. coli*/Coliform Count (EC) plates were used for the enumeration of *E. coli* and coliforms in water sources for handwashing of children (Figure 2). First, 1 mL of the tested water in accordance with the manual was fetched into the 3M Petrifilm. The film was incubated for 24 h at 34–37 °C. After 24 h, total coliforms were counted for both red and blue colonies with gas, while the blue colonies with gas alone were counted as *E. coli*.

Table 1 | The recommended values of the ATP wipe test of Kikkoman Biochemifa Co., Ltd

Purpose of inspection	Place of inspection	First reference value		Second reference value Failure (>)
		Pass (≤)	Careful	
Hygiene education and pre-work hygiene checks	Finger and hand	2,000 RLU	2,001–4,000	4,000
Hygiene management of cooking utensils, etc.	Sink	200	201–400	400
	Refrigerator handle	500	501–1,000	1,000



Figure 2 | Traditional rain water tank (Pieng in Khmer).

RESULTS AND DISCUSSION

Handwashing frequency among rural Cambodian children

Interviews were conducted with a total of 23 children, and parents or older persons answered on behalf of children who were 2 or 3 years old. The gender ratio of the survey subjects was 11 boys and 12 girls (Figure 3). As a result, about 55% of the children washed their hands three or more times a day, but the number of children who never washed their hands was relatively high at 27.5% (Figure 3). The number of daily handwashing frequency among Cambodian boys and girls in the study area is presented in Figure 3, respectively. It can be seen that older children washed their hands more frequently than younger children; it was 6 times/day for girls and 4 for boys. Dajaan *et al.* (2018) also showed that gender affects the correct handwashing behaviour. Our study also showed that girls were highly conscious of handwashing. Previous studies have suggested that handwashing was a means of preventing infectious diseases such as diarrhoea, but it has become clear that in reality, boys and girls in rural areas did not wash their hands sufficiently. In Cambodia, where the under-15 mortality rate is high, the promotion of handwashing behaviour is an important issue. The ATP test results are described in Table 2. The average amount of ATP on hands before handwashing was about 59,000 RLU, which was extremely high compared to the food sanitation control standard of 1,500 RLU (Table 1). In the daily handwashing method, the average ATP value after handwashing was 10,978 RLU, which was decreased by about 74% by washing with water; however, it was still a high value. In addition, as a result of observing daily handwashing methods, the following behaviour was confirmed. This daily handwashing meant that some children used soap and others did not. Moreover, it was observed that some children also wiped their hands with their clothes or used a towel after washing their hands. Previous studies have also stated that handwashing requires soap and a clean towel (Nuki & Kitawaki 2016). It is important that non-reusable towels are needed for drying hands after handwashing. Given the fact that most water sources are heavily contaminated with coliforms, and in some regions, there is water scarcity, WHO published formulations of alcohol-based hand rub (ABHR) which are effective and cheap. A local ABHR production model has been implemented in Uganda (Tusabe *et al.* 2023). Alcohol-based hand rubs have largely been used in healthcare settings; however, Berendes *et al.* (2022) recommended the use of ABHR in community settings to supplement handwashing with soap and water. Therefore, even in rural areas, washing hands with soap and using hygienic towels and alcohol after washing hands is necessary. In addition, in rural areas, people washed their hands near hand pumps and water jars, so it was necessary to consider appropriate handwashing methods that matched their lifestyles. In this survey, the handwashing rate of young children was low and children who did not receive education accounted for about 50% of the total. They have not yet received handwashing education at primary school. They might not have received hygiene knowledge from their parents, relatives or siblings. It seemed that education for children was simply necessary, but hygiene education for adults such as parents was also necessary at the same time. This study suggests that hygiene education for young children is necessary. As a result, we expect that it would lead to ‘C to P’ (Child to Parents), in which hygiene education spread from children to adults.

The gender ratio of this survey subjects													
Age		1-4 years old	5-9 years old	10-14 years old	Total								
Gender	Boy	4	5	2	11								
	Girl	1	8	3	12								
Number of Daily Washing Hands Frequency among Cambodian children in the study area (n = 23)													
Daily Hand washing Frequency		0 time	1 time	2 times	3 times	Over 4 times							
Children Population Rate (%)		27.5	6.8	10.3	37.9	17.2							
Number of daily washing hand Frequency among Cambodian boys in the study area (Boys)													
Age		2	3	3	3	5	6	8	8	9	12	13	
Washing Hands Frequency		3	3	4	2	0	3	0	3	0	0	4	
Number of daily washing hand Frequency among Cambodian girls in the study area (Girls)													
Age		4	5	5	7	7	7	7	8	10	10	10	12
Washing Hands Frequency		3	1	2	3	3	3	3	1	0	3	6	6

Figure 3 | Interviewed children's information and HW situation.

Water use practice and microbial quality of water sources for handwashing

A survey was conducted at children's homes about their water usage patterns (Table 3). At least one well has been installed on the premises of the residents and was used for washing hands, washing and cooking. A survey of seasonal water consumption patterns revealed that during rain seasons, people used the water source in rainwater tanks for handwashing. In Cambodia, traditional rainwater tanks (Figure 2) were used to collect rainwater for various purposes. It was reported that these rainwater tanks were cleaned regularly, but in fact, dust, leaves and garbage might have been on the roof and rainwater collector before it was stored in traditional tanks. This water might not be safe because the color of the water was not transparent. As a result, coliforms were detected in 52 out of 54 rainwater tanks. In addition, *E. coli* was detected in 35 rainwater tanks, which meant that about 65% of the rainwater tanks were contaminated (Table 4). The interviews revealed that rainwater jars were used as water storage tanks during the dry season because it does not rain. Since groundwater was pumped up and/or river water was purchased, these water sources were stored in rainwater jars. The water source in rainwater jars might be a combination of

Table 2 | Result of ATP measurement of washing hands before/after

Gender (1: Male, 2: Female)	Age (years)	Education level	Washing hands frequency	ATP before WH (RLU)	ATP after WH (RLU)	Reduction rate (%)
1	2	0	3	166,765	6,580	96.05
1	3	0	3	170,112	23,589	86.13
1	3	0	4	27,093	11,620	57.11
1	3	0	2	17,851	4,597	74.25
2	4	0	3	26,853	10,637	60.39
1	5	0	0	37,499	5,547	85.21
2	5	0	1	61,663	10,865	82.38
2	5	0	2	13,485	6,559	51.36
1	6	2	3	26,621	7,960	70.1
2	7	1	3	210,396	23,905	88.64
2	7	0	3	7,247	3,765	48.05
2	7	0	3	23,250	5,432	76.64
2	7	0	3	41,602	13,919	66.54
1	8	2	0	75,649	40,504	46.46
1	8	3	3	9,363	7,707	17.69
2	8	0	1	17,499	3,324	81
1	9	2	0	46,763	14,224	69.58
2	10	3	0	108,823	21,893	79.88
2	10	3	3	33,829	4,247	87.45
2	10	0	6	100,600	5,104	94.93
1	12	3	0	76,644	13,743	82.07
2	12	0	6	32,197	5,160	83.97
1	13	5	4	32,503	1,624	95

RLU, relative light unit.

Table 3 | Water use practices in Kandal Leu Village (%)

Water sources by use	Season	Tube well	Surface water	Rain water	Purchasing water
Handwashing	Dry season	46	13	18	23
	Rain season	37	8	50	5
Drinking water	Dry season	9	14	36	41
	Rain season	2	10	76	12
Cooking	Dry season	9	14	34	43
	Rain season	3	10	72	15
Rice cooking	Dry season	9	14	34	43
	Rain season	3	10	72	15

Table 4 | The number of polluted rainwater tanks

Contents	Number of rainwater tanks	Number of polluted tanks	Detection rate (%)
Coliform	54	52	96
<i>E. coli</i>	54	35	65

groundwater, surface water and/or rainwater. In order to use water hygienically from rainwater jars, safe water management, such as regular washing and sterilization using sodium hypochlorite, was required. In addition, it was necessary to take measures such as using a cover to prevent mosquitoes from laying eggs and fallen leaves from entering the jar, and boiling water before drinking. Furthermore, it was necessary to treat properly when using groundwater because it was contaminated with arsenic (Phan *et al.* 2010) and other chemical contaminants (Phan *et al.* 2019, 2023; Sao *et al.* 2023). The present study suggested that handwashing was not frequently being done in the surveyed villages. The COVID-19 pandemic may have changed handwashing behavior, but the infrastructure of water needed for handwashing may not have improved. Looking at developing countries as a whole, other viruses such as the Ebola virus are also still a problem. According to a previous study, the use of alcohol disinfectants like ABHR is better for hand hygiene (Prabhu *et al.* 2023). Although it is important to wash hands, alcohol is also effective for hand hygiene. It is used in medical settings, but is also available anywhere due to the COVID-19 pandemic. Therefore, the use of ABHR in the community, to supplement handwashing with soap and clean water, is recommended. There is a need for a global effort to improve water, sanitation and hygiene with a focus on hand hygiene to mitigate the still-emerging COVID-19 (MacLeod *et al.* 2023). However, further study is warranted because the present study has some limitations; the sample size was not large enough to represent rural children in Cambodia and we did not apply any inferential statistics in our data analysis.

CONCLUSIONS

The present study investigated handwashing behaviour among children under the age of 15 years in a rural village of Cambodia. As a result, approximately 27% of children in rural study areas were found not to wash their hands and 17.2% of children washed their hands more than four times a day. Approximately, 74% of the dirt could be removed from hands by washing with water. Concurrently, it was found that water sources for handwashing differed between the rainy season and the dry season; they used polluted rainwater for handwashing in the rainy season. Approximately, 96% of water from traditional rainwater tanks (Pieng) used for handwashing was contaminated with coliform, while 65% was contaminated with *E. coli*. Therefore, water sources for children's handwashing were undoubtedly not safe. In this study, the handwashing behaviour of children under the age of 15 years and the quality of the water sources for handwashing before COVID-19 have been documented. Appropriate handwashing requires safe water and educational programs on personal and environmental hygiene, for children and their parents. Moreover, ABHR is necessary for hand hygiene. A further study on handwashing should be conducted including the changes in children's handwashing and environmental hygiene behaviour after COVID-19 in rural Cambodia, frequency of ABHR use and time and place of handwashing.

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CONSENT TO PUBLISH

All authors read and approved the content of the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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