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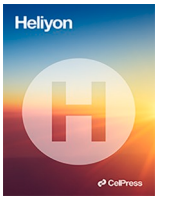

Research article

Outbreak of waterborne acute diarrheal disease in a South District village of Tripura: A public health emergency in the Northeast region of India

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A R T I C L E I N F O

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A B S T R A C T

Food and waterborne outbreaks are a neglected public health problem in India. However, it is important to identify the source of infection and the causative pathogen to curb the outbreak quickly and minimize mortality and morbidity. A retrospective descriptive study was conducted with a line list of 130 diarrheal cases. Epidemiological investigation and laboratory investigation were done. Data were collected from hospital case report forms as well as interviewed affected cases. A case of acute diarrheal disease was reported among the people in the village with abdominal pain, vomiting, and diarrhea from December 31, 2022 to January 3, 2023. Out of a total of 130 recorded cases, 33 stool samples were collected and were positive for Enteroaggregative *Escherichia coli*, *Shigella flexneri* 3a, and *Shigella sonnei* by cultural and molecular tests. The presumptive fecal pollution indicator assay indicated high coliform counts in the water samples (most probable number [MPN]-05) and the presence of *Escherichia coli*. The identified pathogens showed susceptibility to gentamicin and meropenem. People who used public drinking water were found to be infected with acute diarrheal disease (ADD). Quick identification of the causative pathogens and their antimicrobial resistance pattern helped correct antibiotic prescriptions and quick recovery of the patients without any deaths. Thus, a timely implementation of food and waterborne outbreak investigation is crucial to saving lives and preventing the spread of infection.

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1. Introduction

Acute diarrheal disease (ADD) outbreaks pose a significant public health challenge in India, primarily affecting children due to food or waterborne infections [1]. Prompt intervention is crucial, especially in rural areas with limited medical resources for diagnosis and treatment [1]. The World Health Organization (WHO) estimates that globally, nearly 1 in 10 people, or approximately 600 million individuals, suffer from food and waterborne illnesses annually [2]. In India, food and waterborne illnesses and ADD accounted for nearly half of all reported outbreaks between 2011 and 2015, as reported by the Integrated Disease Surveillance Programme (IDSP) [3]. Contaminated water has been a major source of the diarrheal outbreaks in India [4–7].

A significant challenge lies in the fact that the actual extent of food and waterborne diseases remains unidentifed, as many individuals with mild, short-duration symptoms do not seek medical care. Even those who do seek healthcare, mostly do not undergo laboratory tests to identify the specific cause, and not all cases are reported to the health system [8].

Epidemiological investigations have revealed that bacterial pathogens such as *Staphylococcus aureus*, *Salmonella* spp., several pathotypes of *Escherichia coli*, *Vibrio* spp., and *Yersinia enterocolitica* have been responsible for food and waterborne disease outbreaks in India [9]. Notably, the North-Eastern (NE) region experiences frequent food/waterborne disease outbreaks due to diverse sociocultural and food habit patterns, especially during festivals, ceremonies, or rainy seasons. However, research and reporting on such outbreaks in this region are limited.

To address this gap, the Indian Council of Medical Research (ICMR) initiated the 'ICMR-FoodNet' task force (TF) project to identify major circulating food and waterborne pathogens in the NE region and investigate outbreaks, thereby informing public health authorities. Agartala Government Medical College, Tripura (AGMC) is one of the sites in this ICMR-FoodNet project. The main objective of the current ADD outbreak investigation is to identify the causative organism and source of infection so that mortality and morbidity can be reduced and the outbreak can be controlled quickly. This article reports an ADD outbreak in a village in Tripura, marking a significant contribution to understanding and addressing this public health concern in the NE region.

2. Materials and methods

Outbreak setting: The ICMR has initiated a TF project in 2020, including four North East States, i.e., Assam, Tripura, Arunachal Pradesh, and Sikkim. One of the important objectives of this TF project is food and waterborne outbreak investigation. Each team was trained by the ICMR - National Institute of Epidemiology (NIE) team for outbreak investigation. Ethical approval was obtained by the Institutes Ethical Committee (Ref. No 4 (6–11). AGMC/Medical Education/Ethics com/2018/27,980, dated 25th Sept. 2019). On January 1, 2023, the AGMC team was informed by the IDSP team that an ADD outbreak occurred in East Kalabaria village, situated in the South Tripura district, which is one of the study sites. The district is served by twenty-eight public hospitals. An outbreak was confirmed by the IDSP team, and the study was conducted jointly by the IDSP and AGMC teams from January 2-3, 2023. The team visited the affected village and nearby district hospitals for active case searching. The village where the outbreak occurred has a common pipeline water supply through a public water distribution system. Only a few wealthy houses have an additional water supply through tube wells.

Study type: As all affected cases were identified within the village, thus a retrospective descriptive study was conducted to investigate this ADD outbreak. This community-based study encompassed data collection both from the community and hospitals, as well as laboratory investigation to comprehensively analyze the outbreak.

Data collection: From January 2, 2023, a dedicated outbreak investigation team from AGMC, commenced the collection of patient data within the affected community. Active case search was made in the community and interviews were conducted with admitted and non-admitted patients using a standard predefined questionnaire (Case Report Form, CRF) to capture demographic information, while on-site clinical evaluations were made as part of the comprehensive data collection process. Data of age, sex, signs, symptoms, date of onset of illness, date of admission, food intake history, date of discharge, treatment given, and outcome were recorded. A case is defined as an individual suffering from acute watery/bloody diarrhea in the village presented with abdominal pain, vomiting, and/or diarrhea from December 31, 2022 to January 3, 2023. Acute watery diarrhea is defined as the passage of loose and liquid stool more than two times a day [10]. Data was collected from all the affected individuals through interviews and from hospital CRF. An environmental survey was done to investigate the source of infection.

Collection and transport of samples: After getting the informed consent, 33 stool samples were collected from the hospitalized ADD patients, and carefully transported to the laboratory in Stuart's Transport medium using an ice chest [10]. The stool samples were collected one day after the administration of 1st dose of amikacin. Due to the severity of the illness, antibiotic treatment was prioritized during this outbreak. To detect the fecal coliform indicators, environmental sampling was conducted before and after decontamination procedures by collecting four water samples from two locations within the water supply area; one from the storage site of river water and another from the storage site of purified water. These samples, each contained in 100 ml sterile McCartney bottles, were transported to the laboratory within 2 h, maintaining the necessary cold chain protocol.

Processing of the samples and laboratory investigation: The stool samples were initially examined to detect the presence of parasites. Subsequently, these samples were subjected to two different inoculation processes: one in enrichment broths and direct plating on selective media such as MacConkey agar, xylose lysine deoxycholate (XLD) agar and thiosulphate citrate-bile salt sucrose (TCBS) agar and incubated at 37°C. After an overnight incubation period, if turbidity was observed in the enrichment broth, further steps included inoculation onto selective agar media [10]. Upon the growth of isolated colonies, identification was carried out through a combination of biochemical tests and the use of the Vitek 2 Compact system (Biomerieux, France), following the manufacturer's protocol. Additionally, antimicrobial sensitivity testing was made using Kirby Bauer's method using specific discs [10]. The collected

water samples underwent a coliform count analysis using the Most Probable Number (MPN) method. Any samples showing an MPN value greater than zero underwent further culturing after the enrichment process.

Confirmation of diagnosis: The presumptively identified bacterial strains were confirmed by the ICMR-National Institute of Cholera and Enteric Diseases (NICED), Kolkata, which is an external quality assurance services (EQAS) center in the TF study. This confirmation process involved a combination of serological and molecular diagnostic techniques [10].

Data analysis: The data obtained were analyzed utilizing Microsoft Excel, Office 365 version. A line listing of all affected patients was provided by the District Surveillance Officer, from the IDSP team, and an epidemiological curve was created to discern the outbreak's characteristics.

3. Results

3.1. Descriptive epidemiology

The population of the affected village is 14,371, with 7653 (53.2 %) males and 6718 (46.7 %) females. A total of 130 affected cases were interviewed, and a detailed line list was compiled. Analysis of the case data from the line list revealed a noteworthy trend; nearly one-third of the cases (33%) fell within the age group of 0–20 years, suggesting a higher susceptibility of younger individuals (Table 1). In terms of gender distribution among the cases, males (57.7%) exhibited a slightly higher prevalence compared to females (42.3%) with an attack rate of 0.98% in males and 0.82% in females (Table 2).

An epidemic curve revealed a gradual increase in reported cases within the village, spanning a period of 4 days from December 31, 2022, to January 3, 2023. Remarkably, all cases emerged within a week and abruptly ceased by January 4, 2023. This pattern strongly suggests a common-source infection with continuous or repeated exposure for some time, followed by an abrupt reduction in the number of cases due to quick corrective measures undertaken by the state health team. All the reported cases originated from the East Kalabaria Gram Panchayat village, which shares a common public water distribution system, as illustrated in the spot map (Fig. 1). The epidemic curve, derived from the 32 cases of ADD, is depicted in Fig. 2. Notably, out of the total 130 cases, 33 (37.7%) required hospitalization in nearby local health facilities. It is worth highlighting that all cases received appropriate medical treatment, and there were no fatalities due to the quick generation of laboratory reports of causative pathogens, including their antimicrobial resistance (AMR) results. Furthermore, the epidemic curve reveals that the majority of admitted patients exhibited acute diarrhea symptoms between 6 p.m. and 9 a.m. on January 1, 2023, persisting until 12 p.m. on January 3rd (Fig. 2). In the hospital, a total of 33 admitted cases were interviewed, and a detailed case list was compiled. A descriptive outcome of the symptoms among the cases admitted to the health facility is shown in Table 3. All the cases presented with diarrhea, followed by pain in the abdomen (84.8%) and blood in the stool (81.8%).

3.2. Laboratory results

Among the 33 stool samples collected during the study, laboratory analysis using microscopy, culture, and molecular methods revealed 2 samples positive for Enteroaggregative *Escherichia coli* (EAEC), 8 samples positive for *Shigella flexneri* 3a, and 1 sample positive for *Shigella sonnei*. EAEC was identified by polymerase chain reaction (PCR) using specific primer sets (Table S1). Out of 4 samples collected from the under 5 yrs age group, 3 were infected with *Shigella* spp., and in another child, no pathogen was isolated. These findings provided conclusive evidence that the outbreak of ADD was attributed to EAEC and *Shigella* infections. Additionally, 4 water samples were collected from various points within the public water distribution system in the village indicating the presence of coliform organisms, (MPN-05). *E.coli* was also identified from these water samples.

Antibiotic sensitivity testing showed that both the EAEC and *Shigella* spp were resistant to ceftriaxone, ciprofloxacin, cefepime, azithromycin, and tetracycline (Table 4). However, these organisms demonstrated sensitivity to gentamicin and meropenem. These findings proved helpful for clinicians in making timely decisions regarding the use of appropriate medications for treating patients.

Case management: The ADD cases were managed in a nearby district hospital. Patients were prescribed Oral rehydration solution (ORS), gentamicin, and intravenous fluid (IVF). All cases were discharged by January 6, 2023.

Environmental issues: In the village public water distribution system, pipeline water was the main source of drinking water. In addition, few houses have tube wells. High MPN values of coliforms indicated the fecal contamination of the supply water facility through the pipeline with a public distribution system. Leakage in the supply water system resulted in the contamination of water. The

Table 1
Age group distribution of the admitted patients from whom samples were collected.

Age group (years)	Numbers of Cases (n = 33)	Percentage (%)
0–5	4	12.1
6–10	0	0
11–20	7	21.2
21–30	7	21.2
31–40	3	9.1
41–50	5	15.1
51–60	3	9.1
>60	4	12.1

Table 2
Attack rate based on gender.

S.No.	Gender	Population	Cases	Attack Rate (%)
1	Male	7653	75	0.98
2	Female	6718	55	0.82

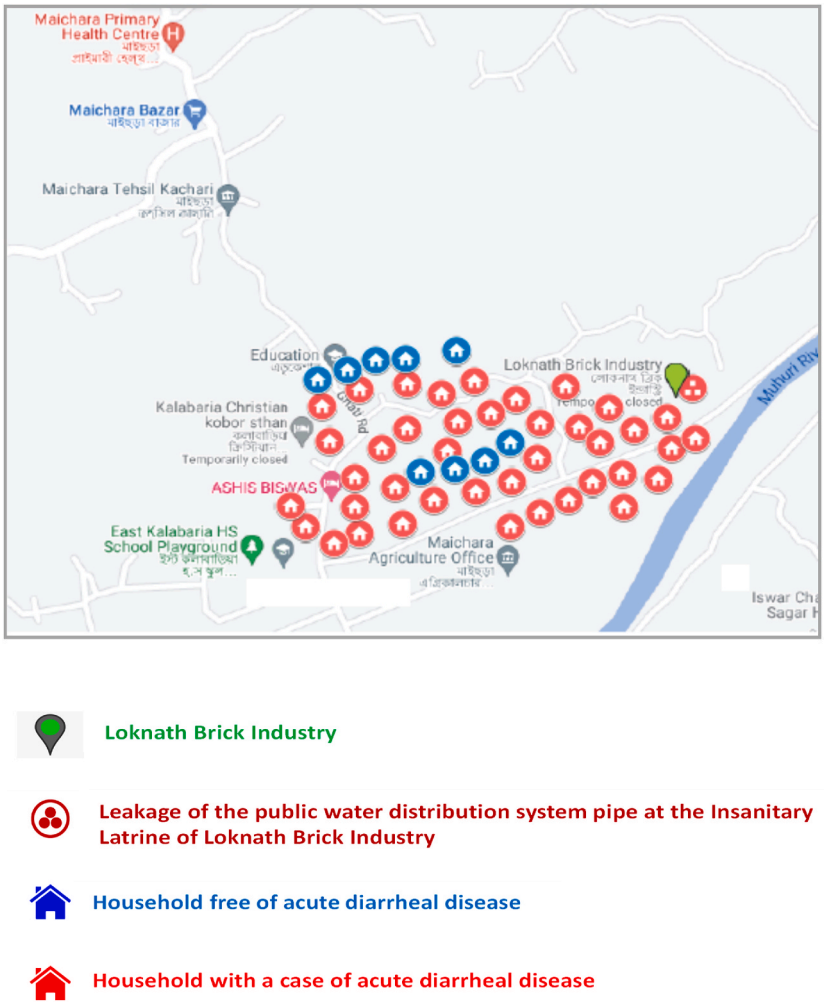

Fig. 1. Spot map of ADDs outbreak in East Kalabaria Gram panchayat showing the Point Source contamination of the public water distribution system.

leakage in the public water distribution system was identified near a local brick kiln, situated adjacent to an unsanitary public toilet.

4. Discussion

ADD outbreaks in India are a persistent public health challenge, imposing a significant burden on both healthcare systems and communities. The country has experienced numerous outbreaks, often with severe consequences, particularly among vulnerable populations. According to the WHO, India has consistently ranked among the nations with the highest incidence of diarrheal diseases. In 2017 alone, India recorded approximately 108,000 deaths among children under the age of five due to diarrhea, highlighting the gravity of the issue within the nation's healthcare system [11].

The Eastern parts of India are vulnerable to ADD outbreaks, particularly in a population of children under 5 year age group[12]. Kolkata, as a densely populated metropolitan city, has experienced several episodes of ADD outbreaks, often linked to contaminated water sources and overcrowded urban areas [13]. In the NE states, ADD outbreaks represent distinct challenges compounded by factors such as remote and hard-to-reach regions, coupled with gaps in healthcare access, increase the population's vulnerability. Moreover,

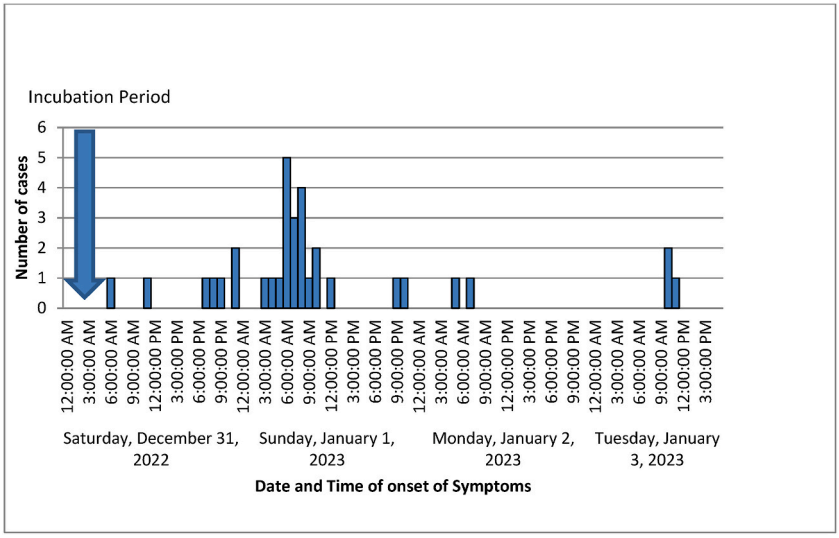

Fig. 2. Epidemic curve of acute diarrheal diseases based on the details of admitted patients from whom samples were collected.

Table 3
Symptoms distribution among the cases admitted to the health facility.

Symptoms	Frequency (n)	Percentage (%)
Nausea	18	54.5
Vomiting	09	27.2
Pain abdomen	28	84.8
Diarrhea	33	100
Blood in stool	27	81.8
Fever	08	24.2

Table 4
Antibiotic sensitivity pattern of the isolated pathogens.

Pathogen	Antibiotic Sensitivity Pattern	
	Resistant	Sensitive
Enteroaggregative *Escherichia coli*	CTR,CPM,AZM,CIP,TET	MRP,GEN
Shigella flexneri 3a & *Shigella sonnei*	CTR,CPM,AZM,CIP,TET	MRP,GEN

Abbreviation; CTR-Ceftriaxone, CIP-Ciprofloxacin, CPM-Cefepime, AZM-Azithromycin, GEN-Gentamycin, MRP-Meropenem, TET-Tetracycline.

monsoon-related flooding can exacerbate the spread of waterborne diseases, including diarrhea [14]. This highlights the need for preparedness and timely response in the face of such multiple factors.

S. flexneri and *S. sonnei* are the important pathogens causing the diarrheal outbreak in India [15]. Large outbreaks of *S. dysenteriae* type 1 have been reported, including the 1984 outbreak in West Bengal and Tripura, which affected over 350,000 people with 3500 deaths [16]. The appearance of ciprofloxacin-resistant *S. dysenteriae* type 1 aggravated the issue of AMR [17]. Outbreaks caused by other *Shigella* species, such as *S. flexneri* and *S. sonnei*, are becoming more widespread in India, as seen in West Bengal (2007), Kerala (2009), and Maharashtra (2010) [18]. These epidemics showed various transmission roots for *Shigella* infection, which include, person-to-person transmission, polluted water sources such as pipelines, and contaminated foods through local cuisines and religious meals [19].

In the present study, analysis of biological samples collected from the affected cases unequivocally attributed the outbreak of ADD to EAEC and *Shigella* spp. The manifestation of symptoms, including diarrhea and abdominal pain, was consistent with the *E. coli* infections. In addition to *S. flexeneri* and *S. sonnei*, an EAEC pathogen was identified during our investigation in the South District village of Tripura. Identification of *Shigella* spp is a big concern in this outbreak. It is known that these bacteria are the source of shigellosis, which causes the invasive type of diarrheal disease or dysentery, as evidenced by the presence of blood in the stools [20]. The combination of these microorganisms highlights the difficulty in tracking outbreaks of waterborne illnesses in this area and emphasizes the importance of strict control and monitoring. Both EAEC and *Shigella* spp. are well-documented pathogens associated with bacterial-induced diarrhea [4,5,7,20–23]. A different study demonstrated that EAEC was frequently responsible for waterborne

outbreaks, demonstrating its adaptability to various environmental factors [21]. Identification of different pathogens from patients' stools as well as from water samples highlighted that the cause of the outbreak was due to fecal pollution of the potable water. Younger age groups, especially children are more affected due to less exposure to diarrheagenic pathogens resulting lack of protective antibodies [21].

Detection of enteric pathogens in this study emphasizes the necessity of focused interventions and extensive water quality monitoring to lower the risk of waterborne illnesses. Continued health surveillance and identification and characterization of pathogens are essential to comprehend the spread of food/waterborne pathogens and to make proper preventative measures, [20,22]. Our study underlines the significance of collaboration across various domains to address water quality issues and protect public health in locations where these illnesses are widespread. It also adds to the increasing body of evidence about the frequency of pathogens associated with waterborne epidemics.

In this investigation, there was no evidence of a common food source implicated in the outbreak, suggesting an alternative route of diarrheal disease transmission. The pipeline water was contaminated due to a leak in the public water distribution system that occurred near a local brick kiln situated adjacent to an unsanitary public toilet. The leakage point was identified and repaired. Prompt repair of the public water distribution system by the local authorities and the implementation of drinking water purification measures through chlorination played a pivotal role in effectively containing the outbreak. Collaboration with local administrative authorities and the Panchayati Raj Institution (PRI) was instrumental in identifying the potential source of infection responsible for this localized outbreak. These findings strongly indicated the likelihood of fecal contamination in the local public water distribution system, serving as the root cause of the ADD outbreak within the community. Laboratory analysis results further substantiated the presence of fecal contamination in the water supply. After a detailed outbreak investigation, the following recommendations were made to the district health authorities:

a) Immediate correction of damaged water distribution pipe.
b) Initiate quick drinking water purification by chlorination and usage of boiled water at the household level.
c) Regular inspection of the water pipeline for any leakage/damage and water quality monitoring

Swift actions, such as the prompt repair of the public water distribution system by the local authorities and the implementation of drinking water purification measures through chlorination, played a pivotal role in effectively containing and mitigating the outbreak very quickly.

4.1. Limitation of the study

Outbreak investigation is always a challenging task. The absence of consent for stool specimens from controls is a major limitation of the study. Also, the collection of water samples from tube wells was not taken for testing.

5. Conclusion

Despite the improvement of hygiene and sanitation through the initiation of "Swachh Bharat Abhiyan" (Campaign Clean India), "Mission Indradhanush" (vaccination coverage of children and pregnant women) "WASH" (water, sanitation, and hygiene) program by the Government of India, ADD outbreak is still a public health problem. Diarrheal outbreak is a major cause of under 5 years of child mortality in developing countries. Every outbreak with two or more epidemiologically related patients needs to be investigated so that the outbreak can be curbed quickly and mortality can be voided. Evidence-based results also help to generate strong policy decisions toward control measurements for the benefit of public health.

The investigation underscores the critical importance of maintaining the integrity of water distribution systems and the necessity for timely intervention to prevent fecal contamination, thereby safeguarding public health and preventing outbreaks of waterborne diseases.

Ethics approval and consent to participate

This study was reviewed and approved by the Institute Ethical Committee (Ref. No 4 (6–11). AGMC/Medical Education/Ethics com/2018/27,980. All participants provided written informed consent to participate in the study.

Data availability statement

All data underlying the results are available as part of the article and no additional source data are required. Data supporting reported results can be found at www.icmrfoodnet.in.

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CRediT authorship contribution statement

Tapan Majumdar: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hritusree Guha:** Writing – review & editing, Writing – original draft, Software, Investigation, Data curation. **Amar Tripura:** Writing – review & editing, Writing – original draft, Validation, Project administration, Investigation, Formal analysis, Data curation. **Bitan Sengupta:** Writing – original draft, Supervision, Investigation, Data curation. **Anup Kumar Ojha:** Writing – review & editing. **Samaresh Das:** Software, Resources, Formal analysis, Data curation, Conceptualization. **Goutam Chowdhury:** Writing – review & editing. **T. Ramamurthy:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Madhuchhanda Das:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31903.

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