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Quantification of Pathogenic Microorganisms in River and Groundwater Samples at Riverbank Filtration (RBF) Site

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ABSTRACT. There are numerous methods for counting the number of bacterial cells in the study of microbiology. The techniques include direct microscopic counts, turbidimetric measurement, and standard plate counts (Viable Counts). The standard approach was chosen for this bacterial enumeration because it is simple to use and significantly more sensitive than turbidimetric testing. A plate count is typically performed by dilution of the original sample, 1 ml transfer onto an agar plate, and even distribution across the plate's surface. Colonies emerged when the agar plates were incubated at 37 °C for 24 hours. Colony-forming unit (CFU) can then be computed as the total number of viable cells. To ascertain the presence of *Salmonella*, *Shigella*, and *Escherichia coli* bacteria, samples of river water and groundwater were subjected to water analysis. Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar, McConkey II Sorbitol agar, and Xylose Lysine Deoxycholate (XLD) agar were the four different types of agar used in the spread plate method. All plates underwent a 24 hour incubation period at 37 °C, after which the colony forming unit (CFU) per milliliter was determined.

Keywords: Pathogenic microorganism, Standard plate counts, Riverbank filtration, Water quality.

INTRODUCTION

One natural resource that is necessary for all life on earth is water. Water is a vital natural resource because of its fundamental role in life, quality of life, the environment, food production, hygiene, industry, and power generation (Meays et al., 2004). Oxygen and hydrogen, or H_2O, are two chemical compounds that are combined to form water. 97% of the water on Earth is salty and found in the oceans, and the remaining 3% is freshwater found in streams and rivers. Even if there are more people on this globe, there is still enough freshwater to feed the current population.

Malaysia's Ministry of Health (MOH) is in charge of keeping an eye on the treatment facilities' raw water quality. Malaysia is one of the developing nations that witnessed fast industrialization and urbanization along with the rapid rise in the global population. Because of this, there is a greater need for water supplies, particularly in residential, commercial, and agricultural regions (Mokthsim and Salleh, 2014). Due to river pollution and rising treatment costs, the use of river water could be compromised as a sustainable source of water supply (Lin et al., 2022). Currently, the primary source of public water supply in Malaysia is by having surface water. Unfortunately, several pollutants from landfills, industrial waste, and agricultural sources have contaminated many sources of surface water in Malaysia

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(Baharudin et al., 2021). If no significant adjustment is made to reduce the pollutants, this scenario will put human safety in greater danger.

In order to avoid supply disruption due to pollution and supply safe drinking water to a community, riverbank filtration (RBF) was introduced as a reliable method of natural treatment to increase the quality of water sources. Riverbank filtration is a sustainable and innovative method of obtaining high-quality drinking water by utilizing natural processes. This approach involves the extraction of water from rivers, lakes, or other surface water bodies through wells located in close proximity to their banks (Sandhu et al., 2019). As the water percolates through the sediment and gravel layers of the riverbed, it undergoes a natural filtration process. This filtering action effectively removes impurities, pathogens, and organic matter, resulting in water that is remarkably clean and safe for consumption (Baharudin et al., 2023).

Riverbank filtration is not only cost-effective but also environmentally friendly, reducing the need for energy-intensive water treatment methods. Its widespread adoption has proven to be an essential component of ensuring a reliable and sustainable source of freshwater in various regions around the world, contributing to the preservation of both water quality and ecosystem health. For more than a century, the RBF system has been widely used in Europe, particularly in Germany, in the Rhine, Donau, and Elbe rivers (Gillefalk et al., 2018; Shamrukh and Abdel Wahab, 2008). Countries including Germany, Austria, Hungary, and the Netherlands have been successful in improving drinking water sources via RBF, artificial recharge, or both. In Malaysia, the implementation of RBF is still scarce, although groundwater forms more than 90% of total freshwater resources but less than 2% of total water usage. In the case of Selangor, groundwater is used as a second source of water supply to guarantee clean and clear water use (Mridha et al., 2019).

The surface water system and groundwater system interact with one another. Surface water goes into an infiltration process where it can enter the groundwater system by percolating down through the soil. Streams can be called gaining or losing streams, depending on the season and location (Chen et al., 2013). Whether a stream is gaining or losing, it can be affected by the pumping of a nearby well. Pumping from a well can cause a dip in the water table (potentiometric surface). This drawdown, or cone of depression, represents a negative head gradient and can cause water from a nearby stream to enter the groundwater system, which is also called induced infiltration (Fetter, 2001). At the Jenderam Hilir RBF site, the interaction between the groundwater and the river was identified by using the minimum and maximum groundwater levels of the various boreholes. The strong relationship between the groundwater and surface water was clearly specified when the increase of the water level in Sungai Langat would also increase the groundwater levels in the well. Higher groundwater levels in comparison to the river level indicated that the groundwater contributed to the surface water. Therefore, the aim of this study is to determine the presence of pathogenic microorganisms such as Total Coliform, *Escherichia Coli*, *Shigella,* and *Salmonella* in rivers and groundwater and quantify them accordingly using different types of agars.

The EMB agar is a medium that is both selective and differential, principally employed for the purpose of isolating

and distinguishing Gram-negative enteric bacteria, with a specific focus on individuals belonging to the Enterobacteriaceae family. The EMB agar medium exhibits a selective property against Gram-positive bacteria while also enabling the differentiation between lactose fermenting and non-fermenting microorganisms. Bacterial strains that possess the ability to ferment lactose exhibit colonies that display a distinct dark, metallic green sheen, which can be attributed to the generation of acidic compounds. In contrast, bacterial strains that lack the capacity to ferment lactose generally exhibit colonies that seem pink or colorless in nature. The utilization of this method is widespread in the detection and characterization of fecal coliforms, including *Escherichia coli*, in the assessment of water quality and clinical microbiology (Abu Sini et al., 2023; Some et al., 2021). The MacConkey agar medium is commonly employed in the isolation and identification of enteric pathogens. MacConkey agar exhibits selectivity towards Gram-negative bacteria while suppressing the development of Gram-positive bacteria. The medium is composed of lactose and pH indicators, which serve to distinguish between lactose fermenting microorganisms (pink colonies) and non-fermenting microorganisms (colorless colonies). The utilization of XLD and SS agar as a selective and differential medium is predominantly employed for the purpose of isolating and identifying *Salmonella* and *Shigella* species, which are known to be potential pathogens linked to gastrointestinal illnesses (Tawfik et al., 2022). Xylose and lactose serve as fermentable sugars in XLD agar, while lysine and deoxycholate work as selective agents. Additionally, XLD agar contains phenol red pH indicator and iron salts. On the other hand, SS agar includes bile salts and brilliant green dye as selective agents, lactose as a carbohydrate source, and a neutral red pH indicator. Xylose Lysine Deoxycholate (XLD) agar and *Salmonella shigella* (SS) agar are both examples of specialized media formulated for the selective separation of *Salmonella* and *Shigella*, two prominent pathogens responsible for foodborne and waterborne diseases.

METHODOLOGY

The process consisted of a preliminary investigation and a detailed site investigation of the study area to get an overview so that more detailed planning could be done to ensure smooth operations. This section will discuss the details of the experiment and test that was done to determine the presence of pathogenic microorganisms in river water and groundwater.

The study area was located at Kampung Jenderam Hilir, Dengkil, Selangor, near the Sungai Langat (Figure 1), where the condition of implementing RBF within that area is very conducive. The river water sample was collected by throwing out the tied bucket into the river, letting the water in and then pulling up the ropes of the bucket onto the road surface to take the sample. For groundwater, the sample was extracted from two existing pumping wells, namely MW01 and MW02, that are available within the RBF site. Groundwater lies under the surface of the land, where it travels through and fills openings in the rocks (aquifer), and most of the time, the water is contained in or by a subsurface layer of soil or rock. The sources recharging the supply of groundwater include rain that saturates into the ground and rivers that disappear underground. Because of the many sources of recharge, groundwater may contain

any or all of the contaminants found in surface water as well as the dissolved minerals it picks up underground.

Figure 1. Location of study area.

The plate count method is the common method used for determining the number of colonies present in any sample (Laird et al., 2004). In order to make the calculation for the number of cells easier, dilutions are designed, which are 1/10, 1/100, 1/1000. For 1/10 or 10^{-1} dilution, it can be made by mixing 1 ml of sample with 9 ml of peptone water. This gives the fraction as in Eq. 1.

$$\frac{100\mu L\ of\ sample}{100\mu L\ of\ sample + 900\mu L\ of\ pepton\ water} = \frac{100\mu L}{1000\mu L} = \frac{1}{10} \quad (1)$$

Samples of river water and groundwater were collected from two groundwater wells, namely MW01 and MW02, for 3 days to determine the number and types of colonies present by following the procedures in The Plate Count Method after incubation at 37˚C for 24 hours. After the sample was taken from the site, the sample was tested daily in the laboratory to determine the presence of pathogenic microorganisms by using four different types of agars which are Eosin Methylene Blue Agar (EMB, HiMedia), *Salmonella shigella* Agar (SS, HiMedia), Macconkey II Sorbitol Agar (HiMedia) and Xylose Lysine Deoxycholate Agar (XLD, HiMedia).

Briefly, the river water and groundwater samples were collected by filling the river water into a tied bucket and pumping out groundwater from the aquifer, respectively, from Sungai Langat, Jenderam Hilir. Once in the lab, 1 ml from the bottle containing the water sample is transferred into a tube containing 9 ml of sterilized peptone water. Thus, the concentration of the bacteria in the tube is exactly 1/10 dilution that in the bottle. Next, 1 ml of the dilution is aseptically transferred onto an agar plate and spread evenly over the surface of the agar plate using a sterilized L-

shape spreader until it dries. The agar plates are incubated at 37˚C for 24 hours in an incubator. After 24 hours, the number of colonies shown on the agar was calculated. Plates with more than 300 colonies cannot be counted and are designated too many to count (TMTC). Plates with less than 30 colonies are designated too few to count (TFTC). The number of bacteria present was determined as colony forming unit (CFU/ml) and was calculated using the formula in Eq. 2.

$$cfu/ml = \frac{No.of\ colonies\ \times dilution\ factor}{volume\ of\ culture\ plate} \tag{2}$$

RESULTS AND DISCUSSION

Results from samples of Day 1 (Figure 2) have shown that the number of colonies present in river water has higher values compared to groundwater well 1 (MW01) and groundwater well 2 (MW02). All four types of agars, which are EMB Agar, SS Agar, MacConkey II sorbitol Agar, and XLD Agar, indicated the highest value of colonies present in river water samples, which are 39.0 x 10^2 CFU/ml, 11.0 x 10^2 CFU/ml, 9.0 x 10^2 CFU/ml, and 1.0 x 10^2 CFU/ml respectively. In comparison with the other two types of groundwater samples, which are from MW01 and MW02, the number of colonies present is much lower. For MW01, every four types of agars, which are EMB Agar, SS Agar, MacConkey II sorbitol Agar, and XLD Agar, give the lower values of colonies present, which are 1.0 x 10^2 CFU/ml, 7.0 x 10^2 CFU/ml, 3.0 x 10^2 CFU/ml, and 0 CFU/ml respectively. For MW02, the number of colonies present is also lower, which are 3.0 x 10^2 CFU/ml for EMB Agar, 0 CFU/ml for SS Agar, 10.0 x 10^2 CFU/ml for MacConkey II Sorbitol Agar, and 0 CFU/ml for XLD Agar. From this result, we can conclude that the sample from river water clearly showed a higher number of pathogenic bacteria than from the groundwater samples.

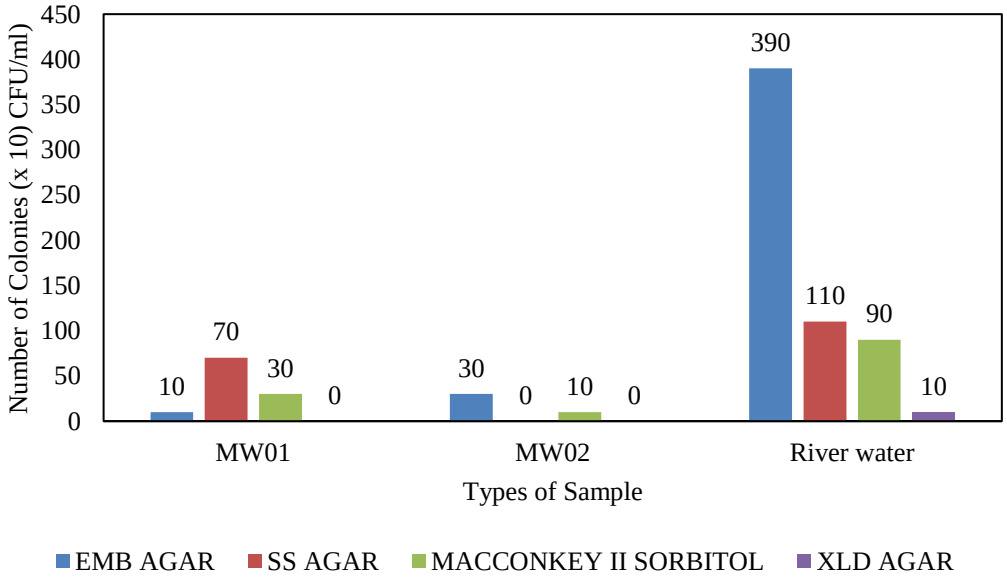

Figure 2. Results of number of colonies (CFU/ml) vs types of samples for day 1.

Next, for Day 2, based on Figure 3, it showed that the number of colonies present in river water also has the highest value compared to groundwater well 1 (MW01) and groundwater well 2 (MW02). However, for Day 2, the average value of all number of colonies present is decreased compared to the average value of colonies present on Day 1. All four types of agars, which are EMB Agar, SS Agar, MacConkey II sorbitol Agar, and XLD Agar, give the highest value of colonies present in river water samples, which are 33.0 x 10^2 CFU/ml, 10.0 x 10^2 CFU/ml, 15.0 x 10^2 CFU/ml, and 0.10 x 10^2 CFU/ml respectively. Different from the other two types of samples, which are MW02 and MW03, the number of colonies present is lower. For MW01, only EMB Agar gives the value which is 0.10 x 10^2 CFU/ml, and others give a value of 0 CFU/ml, which means colonies are not present in SS Agar, MacConkey II sorbitol Agar, and XLD Agar. The same goes for MW02; only EMB Agar gives the value of colonies present, which is 0.20 x 10^2 CFU/ml. The other agar did not show any number of colonies, which is 0 CFU/ml. From this result, we can conclude that the trend for day 2 is significant because from the graph, it can be determined that the value of colonies present on EMB Agar for MW01, MW02, and river water sample is higher which are 0.10 x 10^2 CFU/ml, 0.20 x 10^2 CFU/ml and 33.0 x 10^2 CFU/ml respectively compared to another agar.

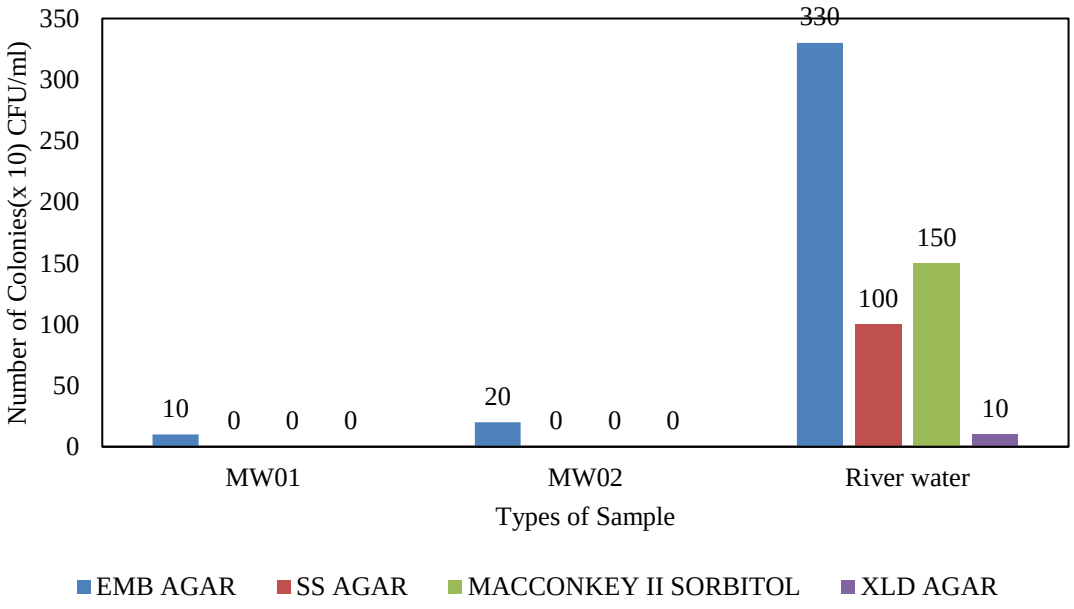

Figure 3. Results of number of colonies (CFU/ml) vs types of samples for day 2.

Lastly, Day 3, as shown in Figure 4, showed similar characteristics to Day 1 and Day 2, where the number of colonies present in river water is the highest compared to groundwater well 1 (MW01) and groundwater well 2 (MW02). However, the number of colonies present in EMB Agar is 24.0 x 10^2 CFU/ml, which is less than the number of colonies present on EMB Agar for Day 1 (39.0 x 10^2 CFU/ml) and the number of colonies present on EMB Agar for Day 2 (33.0 x 10^2 CFU/ml). In the other two types of samples, which are MW02 and MW03, the number of colonies present is lower. For MW01 and MW02, only colonies on EMB Agar are present which are 0.10 x 10^2 CFU/ml for both wells. The other agar did not show any number of colonies which is 0 CFU/ml.

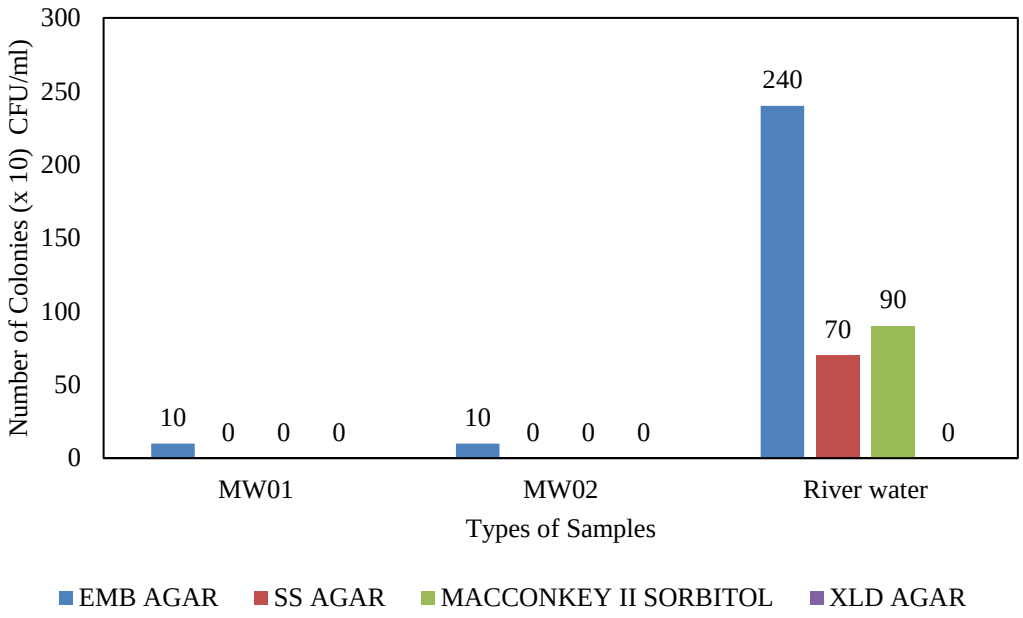

Figure 4. Results of number of colonies (CFU/ml) vs types of samples for day 3

Based on the results for all 3 days, the number of colonies present in river water is the higher number compared to the number of colonies present in groundwater well (MW01) and groundwater well 2 (MW02). For example, for the river water sample that has been tested with EMB Agar, the number of colonies present on Day 1, Day 2, and Day 3 are 39.0 x 10^2 CFU/ml, 33.0 x 10^2 CFU/ml, and 24.0 x 10^2 CFU/ml respectively. From this result, we can conclude that the trend for Day 3 is also significant because, from the graph, it can be determined that the value of colonies present on EMB Agar for MW01, MW02, and river water sample is higher, which are 0.10 x 10^2 CFU/ml, 0.10 x 10^2 CFU/ml and 24.0 x 10^2 CFU/ml respectively compared to other agar.

Based on the overall results, it can be concluded that the Gram-negative bacteria, especially the Enterobacteriaceae, are dominant in each water sample, which shows a high presence of colonies on EMB agar compared to the other agar. The results have also indicated that river water contains more microorganisms due to exposure to too many organic materials such as industrial wastes, animal wastes, agricultural wastes, and solid wastes such as household waste. River water is usually low in mineral content, with high turbidity, taste, and odour that is caused by foreign matter such as organic compounds, inorganic salts, and dissolved gases. Generally, river water has a lot of microbes compared to groundwater. In comparison to groundwater samples, the number of colonies present in MW01 and MW02 is obviously lower compared to the number of colonies present in river water. For example, for the groundwater sample that has been tested with SS Agar, the number of colonies present only on Day 1, which is 0.70 x 10^2 CFU/ml for MW01. The other two days did not show any number of colonies, which is 0 CFU/ml. This scenario shows that groundwater contains less contamination, probably due to the rock and soil becoming a natural filter and removing the contaminants. The water produced might be cleaner and safer to drink as it is filtered down through clay, sand, and rock. In contrast with river water, groundwater lies under the surface of the land, where it travels

through and fills openings in the rocks (aquifer). Groundwater must be pumped from an aquifer to the earth's surface for use. Groundwater has high mineral content, low turbidity, and fewer microbes to digest organic pollutants, less oxygen to sunlight and surface from which organic pollutants can evaporate compared to surface water.

CONCLUSION

The study of pathogenic microorganisms on their presence has been carried out in order to improve our understanding related to the bacteria present surrounding us that cannot be seen through our naked eyes. It is important to determine the presence of pathogenic microorganisms, for example, the presence of microbes in groundwater and river water in order to get clean and safe drinking water without any infections or side effects. The main objective of this study is to determine the presence of pathogenic microorganisms such as Total Coliform, *Escherichia coli*, *Shigella,* and *Salmonella* in groundwater and river water. There are many ways that can be used to determine the number of microbes; one of them is by using The Plate Count Method. This method can possibly be used to determine approximately the number of colonies or microbes that were originally present by growing and counting colonies of coliform bacteria on agar from river water and groundwater samples. This study has also been able to compare the presence of microorganisms from river water and groundwater, meaning which samples have a higher number of colonies present after the laboratory test is done. In order to fulfill the demand and supply safe drinking water to the community, a natural treatment was introduced known as riverbank filtration (RBF). RBF works efficiently and effectively to remove any microorganisms present in the water besides producing high-quality water resources for public supply.

From the result analyses, the following conclusions can be made which are (1) river water has a higher number of colonies present compared to groundwater. River water contains more contamination and has been exposed to too many organic materials such as industrial wastes, animal wastes, agricultural wastes, and solid wastes such as household waste compared to groundwater. Based on the National water quality standard from the Department of Environment, the river water falls into class III, which means extra treatment is required for water supply. In addition, the coliform presence in the groundwater samples was found to be high compared to the permitted range for drinking water, which is absent as specified by the Department of Environment, Malaysia. Therefore, the water produced from groundwater is more likely safe for consumption with additional treatment, and (2) river water has low mineral content, high turbidity, taste, and odour, whereas groundwater has high mineral content, low turbidity, and high Iron (Fe) and Manganese (Mn) but they do not pose significant health risks. Generally, Total Coliform, *Escherichia coli*, *Shigella*, and *Salmonella* are present in river water and groundwater samples with different numbers of colonies.

In conclusion, the objective of this study was achieved, which is first, to determine the presence of pathogenic microorganisms such as Total Coliform, *Escherichia coli*, *Shigella,* and *Salmonella* in groundwater and river water using multiple types of agars for quantification and, secondly, to compare the presence of microorganisms from river

and groundwater. From the result above, it is proven that pathogenic microorganisms are present more in river water with a higher number of colonies presence and a lower number of colonies present in groundwater.

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AUTHOR CONTRIBUTIONS

All authors were involved in sampling and laboratory testing. The contribution in writing for this paper was also done together.

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DATA AVAILABILITY

Not applicable.

COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE WITH ETHICAL STANDARDS

Not applicable.

SUPPLEMENTARY MATERIAL

Not applicable.

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