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TAKRARISHTA A POTENT AYURVEDIC PROBIOTIC: EVIDENCE BASED MICROBIAL STUDY

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ABSTRACT

Background: Ayurveda is an ancient healing science and various medicines and formulations are described under this. Sandhan Kalpana (fermentation process) is a unique dosage form in which acidic and alcoholic fermented drugs are prepared. These drugs were called Asavarishta and are used in various diseases related to gastrointestinal disorders and other diseases as mentioned in Charak and Sushruta Samhitas. Probiotics are live or viable microorganisms that, when consumed, provide health benefits to the patients and normal human beings by maintaining and bringing about the balance of the gastrointestinal microbiota. Evidence of its action against various gut disorders have already been mentioned in various Ayurvedic texts. **Aims and Objectives:** To study probiotic properties of *Takrarishta* using microbial analysis which include preparation of *Takrarishta* as per the Standard Operating Procedures (SOP), analyse the Physico-chemical properties of *Takrarishta* in a designated NABL laboratory following GLPs and to evaluate probiotic bacterial contents of *Takrarishta* using in-vitro cultivation methods. **Materials and Methods:** In the present study, *Takrarishta* has been prepared as mentioned in Ayurvedic Formulary of India and samples are subjected to microbial analysis for the presence of probiotic bacteria. **Results and Conclusion:** *Takrarishta* was found to contain viable bacterial and yeast strains, notably Lactobacillus spp., Bifidobacterium spp., Streptococcus

thermophilus, and Saccharomyce boulardii in par with the quantities required as per WHO criteria and qualified to be called as “Ayurvedic Probiotic”.

KEYWORDS: Asavarishta, Takrarishta, Probiotic, Sandhan Kalpana, gut disorders.

INTRODUCTION

Probiotics are live or viable microorganisms that, when consumed, provide health benefits to the patients and normal human beings by maintaining and bringing about the balance of the gastrointestinal microbiota. The concept of probiotics was first proposed in the early 20th century by *Elie Metschnikoff*.[1] However, the idea gained significant recognition in recent years with the rapid expansion of the functional food industry. India is quickly becoming a key market for probiotic-based foods, with major contributors including Nestlé, Mother Dairy, Danisco, Chr. Hansen, Yakult, and Dano. The increasing globalization of the food sector has further accelerated the availability and consumption of probiotic products in India. Given the widespread claims regarding their health benefits, there is a growing necessity for regulatory measures to ensure product safety, efficacy, and standardization to protect consumers.

The microbiome refers to the collective community of microorganisms, including bacteria, fungi, and protozoa, inhabiting a defined ecological niche such as the gastrointestinal tract of animals including human beings. This complex microbial ecosystem plays a vital role in regulating host physiology, cellular metabolism, and immune functions. Any disturbance in this delicate host–microbiota equilibrium is often correlated to the emergence of several diseases and disorders, including inflammatory bowel diseases, metabolic disorders, and malignancies.[1] Alterations in gut microbial composition have also been associated with autoimmune diseases, allergies, asthma, and metabolic syndromes. Conversely, the presence of beneficial microorganisms contributes to the maintenance of gut health, strengthens the immune response, and potentially reduces disease risk.[2]

According to the World Health Organization (WHO), probiotics are defined as the “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.” Common and effective probiotic species include members of Lactic Acid Bacteria (LAB), Bifidobacterium, Streptococcus thermophilus, and yeast such as Saccharomyces boulardii. For a microbial strain to qualify as a probiotic, it must meet specific criteria. To

achieve beneficial health effects, a minimum daily intake of 10^9–10^{11} CFU (colony-forming units) of viable bacterial or yeast cells is recommended.[3]

The range of commercially available probiotic-enriched food products has expanded considerably over recent decades. The majority of these are dairy-based formulations, including fermented milk, yogurt, cheese, buttermilk, ice cream, and milk powder, with yogurt representing the dominant segment of the global probiotic food market.[4] However, non-dairy applications are also gaining prominence, encompassing soya-based products, nutritional bars, breakfast cereals, and fruit or vegetable juices, all serving as effective delivery vehicles for probiotic microorganisms.[4]

Ayurvedic Concept of Fermentation

Sandhan Kalpana is a unique dosage form in which acidic and alcoholic fermented drugs are prepared, which are widely used for various therapeutic purposes, due to its various advantages such as potency, palatability, quick action, the convenience of administration, and longer shelf life.

Sandhana Kalpana is divided into two types i.e., Madya Kalpana and Shukta Kalpana.[5] Madya Kalpana are said to be alcoholic preparation that called Asavarishta. These preparations are self-generated alcoholic formulations that possess both therapeutic and preservative properties. Whereas Shukta Kalpana referred to acidic preparation. These drugs are used in various diseases related to gastrointestinal disorders and other diseases as mentioned in Charak and Sushruta Samhitas.

In Sandhan Kalpana, Sandhana dravya provides the inoculums to start the fermentation. The process of fermentation necessitates the presence of fermenting micro-organisms known as yeasts. Fermentation fundamentally requires the presence of microorganisms, particularly yeasts, which facilitate the biochemical conversion of sugars into alcohol and other metabolites.

In Ayurveda, substances such as Dhataki Pushpa (Woodfordia fruticosa flowers), Madhuka Pushpa (Madhuca longifolia flowers), Surabeeja (fermenting seeds), and yeast are commonly employed as Sandhana Dravya—the fermenting agents that initiate and sustain the fermentation process.

According to the Charaka Samhita and Sushruta Samhita, Sandhana Kalpana formulations are indicated in the management of various gastrointestinal disorders, as well as a range of systemic ailments, due to their ability to enhance digestion, absorption, and overall metabolic activity.

In this study, *Takrarishta*, a classical Ayurvedic fermented formulation was evaluated for its probiotic potential. *Takrarishta* is mentioned in Charak Samhita Grahanidosha chikitsaadhyay.[6] *Takrarishta* also mentioned in AFI followed the refrence of Charak Samhita.

Properties of Good Probiotics (ICMR Guidelines on Probiotics, Department of Biotechnology, 2010)

1. Resistance to gastric acid
2. Bile acid resistance
3. Ability to reduce pathogen adhesion
4. Antimicrobial activity against potentially pathogenic bacteria
5. Bile salt hydrolase activity

MATERIALS AND METHODS

AIMS AND OBJECTIVES

Aim- To study probiotic properties of *Takrarishta* using microbial analysis.

Objectives

1. To prepare *Takrarishta* as per the Standard Operating Procedures (SOP).
2. To analyse the Physico-chemical properties of *Takrarishta* in a designated NABL laboratory following GLPs.
3. To evaluate probiotic bacterial contents of *Takrarishta* using in-vitro cultivation methods. *Takrarishta* is a fermented medicine and ***Takrarishta*** **is mentioned in charak samhita Grahanidosha Chikitsadhaya.[6]**

Takrarishta contains takra, as drava Dravya. And other ingredients are yavani (fruit), amalaka, haritaki (fruit), maricha, saindhav lavan, Samudra lavan, sauverchal lavan, vida lavan, romak lavan.

Takrarishta **preparation** (Reference charak Samhita; Grahinidosha Chikitsadhay)

Preparation of 3 Samples of *Takrarishta*

Date of starting : 1 june 2024

Date of completion 21 june 2024

Equipments

Glass jar (40 centimeter length, 15 centimeter diameter-mouth, 5 lit capacity), cotton cloth, spetulla, grinder etc.

Identification of all raw drugs was done in the Department of Dravya Guna and Ras Shastra & Bhaishajya Kalpana, Institute for post graduate institute Ayurved, jodhpur. It was done based on characters and parameters mentioned in API.

Table showing ingredients of *Takrarishta*

Serial no.	Ingredient	Latin name	Used parts	Quantity	Quantity in metric
1	Ajamoda	Trachyspermum ammi	Fruit	3 pala	144 grams
2	Amlaki	Phyllanthus emblica	Fruit	3 pala	144 grams
3	Haritaki	Terminalia chebula	Fruit	3 pala	144 grams
4	Maricha	Piper nigrum	Fruit	3 pala	144 grams
5	Saindava Lavana	Sodium chloridum	Saindava Lavana	1 pala	48 grams
6	Vida Lavana	------	Vida Lavana	1 pala	48 grams
7	Samudra Lavana	Sodi muris	Samudra Lavana	1 pala	48 grams
8	Sauvarchala Lavana	Unaqua Sodium Chloride	Sauvarchala Lavana	1 pala	48 grams
9	Romaka Lavana	----	Romaka Lavana	1 pala	48 grams
10	Takra (Cow buttermilk)	Eng- Butter Milk	Takra (Cow buttermilk)	1 adhak	3 litres

All raw drugs were procured from authenticated sources in the specified quantities as per the classical references. Each ingredient was examined for its quality and purity before use.

Identification of all raw drugs was done in the Department of Dravya Guna and Ras Shastra & Bhaishajya Kalpana, Institute for post graduate institute Ayurved, jodhpur. It was done based on characters and parameters mentioned in API.

Procedure of Preparation of Takrarishta

According to Ayurvedic Formulary of India (AFI) Part I, Takra is obtained by adding an equal quantity of water to curd (Dadhi) and then churning it to remove the butter. The remaining liquid is known as Takra.[7]

Takrarishta was prepared with using 3 lit. Takra and 816 gram powdered Aushadh Dravya.

All the specified herbal ingredients were individually powdered to a fine consistency. Three liters of the freshly prepared Takra was taken in a clean, sterilized glass container. The powdered ingredients were added sequentially with continuous stirring to achieve a homogeneous mixture.

The prepared mixture is then transferred into a fumigated glass jar. The jar was tightly sealed, and its mouth was wrapped with a clean cotton cloth layered with clay to prevent external contamination while allowing controlled air exchange. The container is kept in a dark place for fermentation.

Upon confirmation of fermentation completion, the product was filtered through a muslin cloth folded in four layers to extract the liquid from coarse particles and sediments. The filtrate was kept for two days to allow sedimentation.

Subsequent re-filtrations was carried out at two-day intervals to obtain a clear, sediment-free liquid. Three successive filtrations were performed to ensure clarity and purity. The final filtrate, representing *Takrarishta*, was stored in a clean, transparent borosilicate glass jar and kept in a cool, dark environment away from direct sunlight.

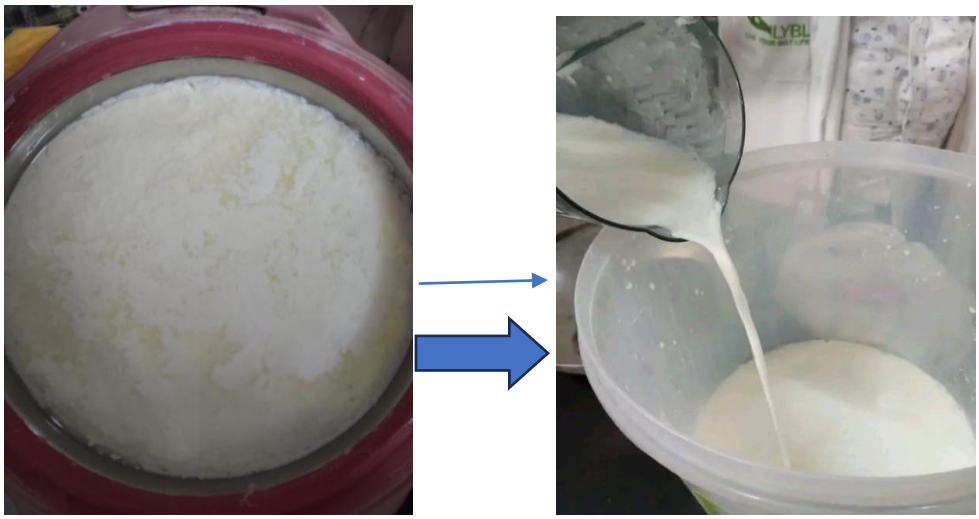
All three samples were prepared with using same method.

No. of sample	Initial amount of Takra	Initial amount of mixture	Obtained amount *Takrarishta*	Loss in the amount of Takra	% of loss
Sample I	3 lit.	3.816 kg	1.515	1.485	49.53
Sample II	3 lit.	3.816 kg	1.525	1.475	49.16
Sample III	3 lit.	3.816 kg	1.530	1.490	49.00

All the samples of prepared *Takrarishta* attained Siddhi lakshana between 18-20 days.
Almost 50 % Takra is absorbed by raw material, so the yield obtained is 50% of the total Takra that was added.

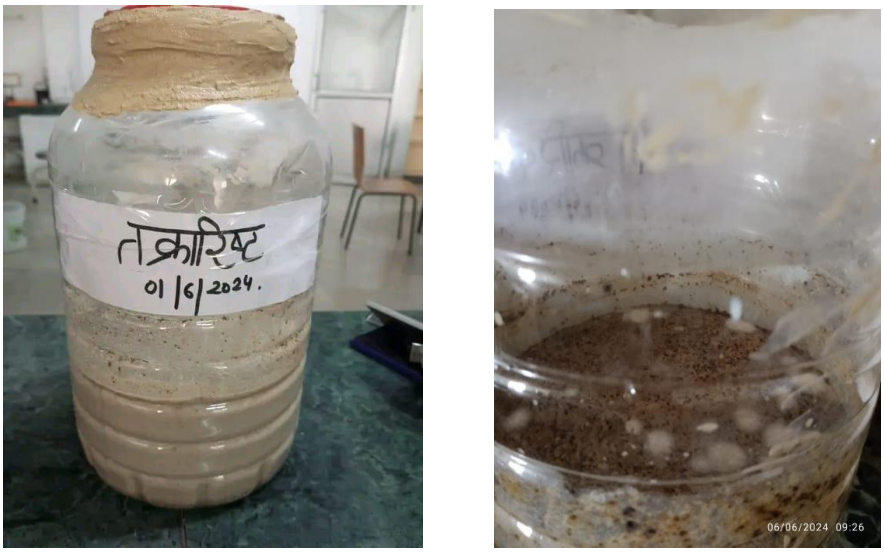
Takrarishta was prepared at Teaching Pharmacy, Department of Rasa Shastra & Bhaishajya Kalpana, G.S. Ayurvedic College, Pilkhuwa, Hapur.

Pictures of pharmaceutical preparation of *Takrarishta*



Dadhi **Takra**

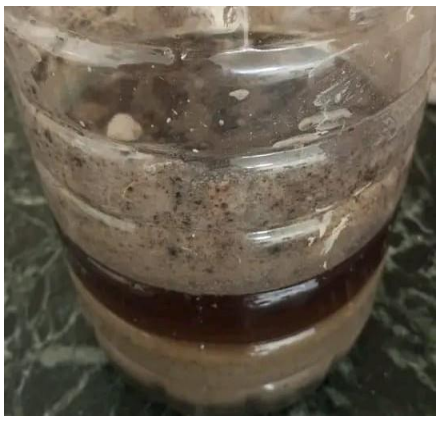
Powder of Aushadh Dravyas ---- mixed all ingredients with Takra and sealed the jar



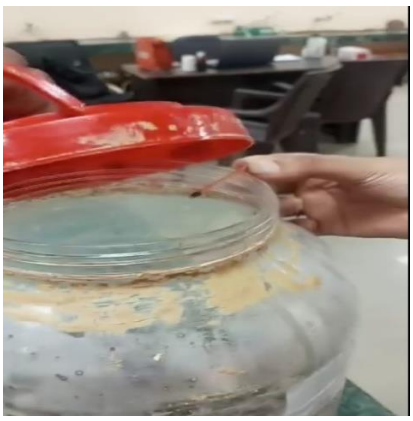
1st June 2024 -Day one **Obeservation on 6th June, 2024**

Observation on 18th june, 2024

Observation on 18th june, 2024



Observation on 21st june, 2024



Flame test on 21st june, 2024

After filteration Sample 1, Sample 2, Sample 3

Physico-chemical Analysis[10] of three samples

Table showing Analytical Report of 3 samples

S. no.	Name of parameter	Sample 1	Sample 1	Sample 1
1	Specific Gravity 1	1.0381	1.0382	1.0381
2	Refractive Index1	1.360	1.363	1.360
3	Ph Value2	3.17	3.18	3.17
4	Viscocity3	1.10	1.12	1.11
5	Total Solids 4	7.67 %w/w	7.69 %w/w	7.67 %w/w
6	Acid Value 4	7.74 mg KOH/g	7.79 mg KOH/g	7.74 mg KOH/g
7	Total Sugar 5	NIL	NIL	NIL
8	Non-Redusing Sugar 5	NIL	NIL	NIL
9	Alcohol value 6	3.4 %v/v	3.45 % v/v	3.47 % v/v
10	Total acidity 7	.77 % v/v	.75 %v/v	.77 %v/v
11	TLC 8 Thin Layer Chromatography Mobile Phase (Toluene:Ethyl Acetate:Formic Acid: 6:4:02)	**Rf Value** – 254nm- 0.03, 0.18, 0.34, 0.38, 0.43 White Light - 0.03, 0.06, 0.18, 0.24, 0.38 After Derivatizaton 366nm – 0.03, 0.18, 0.24, 0.38, 0.97	**Rf Value** – 254nm- 0.03, 0.18, 0.34, 0.38, 0.43 White Light - 0.03, 0.06, 0.18, 0.24, 0.38 After Derivatizaton 366nm – 0.03, 0.16, 0.24, 0.37, 0.99	**Rf Value** – 254nm- 0.03, 0.18, 0.34, 0.38, 0.43 White Light - 0.03, 0.06, 0.18, 0.24, 0.38 After Derivatizaton 366nm – 0.03, 0.17, 0.24, 0.38, 0.99

Microbial Analysis

In the present study, three bacteria (LAB-Lacticacid Bacteria/lactobacillus, Bifidobacterium, Streptococcus Thermophilus) and one yeast from the *Takrarishta* were analyzed for its probiotic characteristics. The probiotic properties of the selected strains were Probiotic.

1. Probiotics are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (FAO/WHO, 2002).[8]
2. The effectiveness of a probiotic is often measured by its "colony-forming units" (CFU), which represents the estimated number of live bacterial cells in a product. CFU values can range from million to billion. CFU is determined in the laboratory because it tells us the viability of the bacteria before they’re exposed to the GI tract.[9]
3. Probiotic activity is often expressed as Colony Forming Units (CFU), representing viable bacterial counts. Generally, probiotic supplements contain 1–10 billion CFU per dose for maintaining gut health, while therapeutic preparations may exceed 50 billion CFU for specific conditions such as IBS or post-antibiotic therapy.

- Supplemental probiotics come in a wide range of CFUs—from the high millions to as many as 200 billion.

The cultivation of probiotic microorganisms requires the use of specific media and incubation environments optimized for each strain to ensure accurate enumeration and viability assessment. Plate count method is used for cultivation of the microbes and medium used for cultivation is given below.

Table showing the various media[11-14] used for microbe cultivation

Microorganisms	Medium	pH	Temperature of Incubation	Conditions	Time References
Lactobacillus	MRS Agar	5.6–5.8	37 ◦C±1◦C 5%	CO2 or anaerobic	72 h ±3 h
	MRS Agar	6.3–6.7	38 ◦C±2◦C	anaerobic	3–5 days
	LAPT Agar	6.45–6.55	37◦C		
Bifidobacterium sp.	TOS-MU	6.5–6.7	37 ◦C±1◦C	anaerobic	72 h ±3 h
	MRS Agar	6.3–6.7	38 ◦C±2◦C	anaerobic	3–5 days
	Blaurock medium, MPC-5	7.0–7.4	38 ◦C±1◦C		4–5 days
	RCM	7.0	37 ◦C±1◦C	Anaerobic	72 h ±3 h
Streptococcus Thermophilus	M17	7.0–7.4	44 ◦C±1◦C	5% CO2	72 h ±3 h
	ST Agar	6.7–6.9	37 ◦C	aerobic	24 h
Saccharomyces boulardii	SDA	5.4–5.8	37 ◦C±1◦C	aerobic	72 h ±3 h

Abbreviations used: MRS Agar— De Man, Rogosa and Sharpe (MRS) agar, ST Agar: Streptococcus thermophilus Agar, RCM : Reinforced Clostridial Medium Agar, SDA: Sabouraud Dextrose Agar, LAPT Agar : Lactobacillus Agar with Peptone and Tween 80, TOS-MU Agar: Transgalactosylated Oligosaccharide – Mupirocin Agar Study of *Takrarishta* for its probiotic properties and microbial analysis was done at Arbro Pharmaceutical analytical lab (NABL), Delhi.

Various Agars are used in the study are as below

Table showing CFU values of selected Miroorganisms, no. of billion CFU per single dose.

S. no.	Microorganism	CFU value in billion CFU/ml per single dose of *Takrarishta*		
		1ml	24 ml	in 48 ml/1 pala
1	Lactobacillus (L. sporogenes)	0.76	18.24	36
2	Bifidobacterium	0.59	14.16	28
3	Streptococcus thermophilus	11.1	266	532
4	Saccharomyces boulardii (yeast)	0.07	1.68	3.2

In ayurveda, Acharya Sharangdhar mentioned one pala/ 48 ml dose of asavarishta *Takrarishta* prepared in June 2024 and analyzed in February 2025 showed the colonies and growth values of various probiotic strains including Lactic Acid Bacteria (Lactobacillus and Bifidobacterium). These species are recognized as Generally Regarded as Safe (GRAS) and are widely used as probiotics.

DISCUSSION

Physicochemical analysis

For Physicochemical analysis including organoleptic examination of *Takrarishta*, the three samples (I, II, III) of prepared drug (*Takrarishta*) were analyzed at S.R. Labs and Research Center, Jaipur Physico chemical analysis of *Takrarishta* showed specific gravity of 1.0381 specifying denser than water, pH value of 3.17 makes it strongly acidic, mildly viscous (value 1.1) with total solids (dissolved and undissolved) value of 7.67%v/v signifying thicker liquid. Similarly, acid value of 7.74 mg KOH/g shows that the prepared drug rich in free fatty acids which helps in increasing drug absorption, with no sugars. Alcohol value of 0.77 % v/v shows smaller amount of alcohol compared to other *Asavarishtas*. TLC values at 254 nm of *Takrarishta* prepared showed values between 0.03 to 0.4 which is responsible for imparting light blue color of the prepared drug.

a) All the 3 samples, are physico-chemical analysis were found to contain close values and showed the nature of *Takrarishta* as denser than water, strongly acidic, mildly viscous with solid value signifying thicker liquid, rich in free fatty acids increasing drug absorption, with no sugars and smaller amount of alcohol compared to other *Asavarishtas*. TLC values at 254 nm of *Takrarishta* prepared showed values between 0.03 to 0.4 which is responsible for detection of alkaloids at light blue color of the prepared drug.

All three samples of *Takrarishta* exhibited similar values consistent with API and PI standards, confirming **pharmaceutical standardization** and reproducibility of process.

Discussion on microiobial Study

As per AFI (Ayurveic Formulary of India) the dose of *Takrarishta* – 12-24 ml.

Acharya Sharangdhar has given one pala (48 ml) dose of Asavarishta.

Table showing CFU values of selected Miroorganisms in different dose.

S. no.	Microorganism	CFU value in billion CFU/ml per single dose of *Takrarishta*			
		1ml	12 ml	24 ml	in 48 ml/1 pala
1	Lactobacillus (L. sporogenes)	0.76	9.12	18.24	36
2	Bifidobacterium	0.59	7.08	14.16	28
3	Streptococcus thermophilus	11.1	133.2	266	532
4	Saccharomyces boulardii (yeast)	0.07	.84	1.68	3.2

CONCLUSION

In line with the WHO definition, probiotic supplements contain 1–10 billion CFU per dose for maintaining gut health. In our study, a single dose of *Takrarishta* was found to contain viable bacterial and yeast strains, notably Lactobacillus spp., Bifidobacterium spp., Streptococcus thermophilus, and Saccharomyce boulardii. These microorganisms contribute to gut health by maintaining microbial balance, enhancing digestion, and providing immunomodulatory effects.

Thus, *Takrarishta* can be considered an "Ayurvedic Probiotic", where the synergistic interaction between herbal phytochemicals and beneficial microorganisms results in a bioactive, functional fermented product. The formulation not only aligns with modern probiotic concepts but also reinforces traditional Ayurvedic understanding of gut health through Sandhana Kalpana.

In view of the results above obtained from our microbial study, *Takrarishta* contains the major bacteria showing the probiotic properties and traditionally been used for gut disorders and qualify to be a potent probiotic. conclude that *Takrarishta* is an "Ayurvedic Probiotic".

Conflict of interest: None.

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Data Availability: data pertaining to the manuscript will be available to the public and provided by them after query or not.

Declaration on Contribution: all authors of a published work have made significant contributions to the research and take responsibility for the accuracy and integrity of the work.

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