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Intestinal microflora on physical performance in athletes – a comprehensive review

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ABSTRACT

Introduction: The intestinal microflora is a crucial component of the human body, developing in parallel with the host and playing a pivotal role in maintaining health throughout life. The composition of the microbiota is dynamic, influenced by factors such as genotype, diet, and initial colonization at birth, resulting in a unique microbiotic profile for each individual. The gut microbiota supports a variety of physiological functions, including immune modulation, nutrient metabolism, and the synthesis of essential vitamins and neurotransmitters. Importantly, it maintains homeostasis, through the bidirectional communication system between intestines and central nervous system, which is necessary for all forms of physical performance.

Purpose of the work: The purpose of this study is to review the effects of intestinal microflora on physical performance and endurance in athletes. Special attention is given to the influence of dietary supplements, which can have an impact on composition of the microflora.

Materials and methods: A comprehensive analysis of research articles available on PubMed, Google Scholar, Web of Science, Embase, and Scopus was conducted using search terms related to: "intestinal microflora," "physical performance," "gut-brain axis," "athletes" and "physical training."

Results: The intestinal microflora is linked to the increased performance during physical exercise in athletes. Understanding this influence on the health and physical ability of athletes will allow better results in competitions and endurance.

Keywords: intestinal microflora, microbiome, physical performance, metabolism regulation, probiotics, prebiotics.

INTRODUCTION

The intestinal microflora is an integral part of the human body. It develops in parallel with the development of the host and maintains its temporal stability and diversity. It plays an important role in the host’s body through adulthood until death. The diversity of microorganisms in the gut is dynamic. Every person possesses a distinct intestinal microbiota, which is determined by genotype, the initial colonization acquired at birth and dietary habits. [1]

The human intestinal microbiota is composed of various microorganisms, including bacteria, eukaryotes, viruses, and parasites. They have adapted to the physical and chemical stresses in the intestine to proliferate rapidly without being eliminated. They have developed the right molecular pattern on the cell surface that can attach the habitat in which they want to live, as well as various enzymes that allow them to utilize available nutrients. [2] The gut environment primarily supports the growth of bacteria from seven dominant groups: *Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria* and *Archaebacteria.* [2, 3]

Human gastrointestinal tract is a large microbial ecosystem that contains several trillion microbial cells. These microorganisms collectively make up what is known as the gut microbiota, and they are essential for maintaining various physiological functions in the human body. The composition of the gut microbiota is influenced by several factors, including genetics, diet, age, environment, and lifestyle. Active individuals often have greater microbiome diversity.

Disorders in the composition of the intestinal microbiota may lead to gastroenterological disorders such as inflammatory bowel disease (IBD), as well as neurological, respiratory, metabolic, liver and cardiovascular diseases. [4]

The intestinal microbiota are continuously involved in modulating the host's immune system, strengthening intestinal barrier and reducing inflammation. It protects against overgrowth of pathogens preventing dysbiosis. [5] It has immunomodulatory functions, regulating cytokine levels through interactions with the gut-associated lymphoid tissue (GALT). It is considered the largest lymphatic organ in the human body. [6]

The microbiome is involved in the synthesis of vitamins, such as vitamin K and from the B group, such as B_7, B_9, and B_{12}. The gut microbiota produces several neurotransmitters that influence brain function and mood. Key neurotransmitters include serotonin, dopamine, GABA, acetylcholine, norepinephrine, histamine, and endorphins. [7] Microbial production of neurotransmitters helps regulate stress, indirectly enhancing the ability to cope with training demands. [7, 8]

These compounds are synthesized by gut bacteria and can affect the central nervous system through the gut-brain axis. Imbalances in the microbiota and neurotransmitter production may contribute to mood disorders, anxiety, and other neurological conditions. Through anaerobic fermentation, gut microorganisms produce short-chain fatty acids - SCFAs, which is the primary energy source for the colonocytes. It modulates gut hormonal functions, affects host's cell proliferation and vascularization.

The gut microbiota plays a crucial role in influencing the central nervous system (CNS) through the bidirectional communication of the gut-brain axis, a dynamic system that facilitates continuous interaction between the gastrointestinal tract and the brain. This connection is built upon several interconnected pathways that include metabolic, hormonal, neural, and immunological processes. [8] Dysbiosis or disruptions in the gut-brain axis can increase fatigue, impair recovery, and worsen the body's stress response, negatively affecting overall physical and mental performance.

Microorganisms can influence CNS functions in multiple ways, including signaling via the vagus nerve, modulating the immune system, regulating the hypothalamic-pituitary-adrenal (HPA) axis, and altering tryptophan metabolism.

Gut microbiota plays a significant role in shaping physical performance. Through the production of short-chain fatty acids (SCFAs), it supports energy metabolism, enhancing the efficiency of nutrient utilization. [9] Gut bacteria also modulate inflammatory responses and muscle recovery after exercise, which are crucial for training adaptation. Studies suggest that greater microbiota diversity is associated with better athletic performance, and an appropriate diet, including probiotics and prebiotics, can enhance its beneficial effects on an athlete's body. Different types of physical activity can alter the diversity and metabolic activity of gut microorganisms. [10]

ROLE IN METABOLISM REGULATION

Athlete’s intestinal microflora differs from individuals with sedentary lifestyle, reflecting adaptations to, among others, their intense training. Studies of recent years indicate that gut bacteria might improve sport performance. Bacteria in the large intestine ferment undigested nutrients, such as non-digestible carbohydrates and ten percent of consumed protein. Products of fermentation are short-chained fatty acids (SCFAs), mainly propionate, butyrate and acetate. SCFAs might then be used as an energy source for muscles, where they are oxidized, resulting in increased performance. [11]

SCFAs may play a role in metabolism regulation. Studies concerning this topic are mainly on in vitro and murine models. In vitro models revealed SCFAs, mainly propionate, induced intestinal gluconeogenesis. 23% of systemic glucose in rats which ingested butyrate was created in intestinal gluconeogenesis. In murial models, glucagon-like peptide 1 (GLP-1) was reported to decrease in exposure to SCFAs, which would lead to increased serum glucose. Increased glucose improves performance and delays exhaustion. [12]

Scheiman et. al examined the connection between *Veilonella genus* and sport performance. Stool samples of runners, collected up to seven days after the marathon, showed an increase in *Veilonella genus*. The stool samples were then injected into mice. Contrary to control, mice with *Veilonella atypica* obtained from the marathon runners were able to run on average 13% longer. Working muscles produce lactate and *Veilonella*, unlike most bacteria, possess enzymes required to metabolize lactate into SCFAs, mainly propionate, which then is taken through intestinal epithelium. [13]

ROLE IN INFLAMMATION REGULATION

Athletes are at risk for stress, both psychological from competing, but also physical, resulting from bodies' response to intense and prolonged exercise. Stress, through increased release of stress hormones, might result in “leaky-gut”, where tight junctions between intestinal cells loosen, increasing intestinal permeability. Loosening of tight junctions during exercise may also occur due to hyperthermia and ischemia of gastro-intestinal tract. This allows lipopolysaccharides (LPS) of bacteria to locate outside the gastro-intestinal tract. When translocated LPS are recognized by CD14 and toll-like receptor 4 (TLR4), they trigger the production of pro-inflammatory cytokines, causing inflammation. Ischemia also enhances the production of ROS, which contributes to a leaky gut. Microbiota might affect inflammation by production of SCFAs. Serving as the main energy source for colonocytes, SCFAs may prevent epithelial degradation from ischemia, keeping intestinal barrier intact and reducing inflammation. [17] Butyrate, which concentration is reported to grow with exercise, not only increases expression of tight junction proteins in colon epithelia but also exhibits anti-inflammatory effects by regulating neutrophil function and adhesion. It might also modulate immune cells proliferation and apoptosis. Butyrate was reported to decrease the proinflammatory cytokines IFN-γ, TNF-α, IL-1β, IL-6, and IL-8. [18]

Aside from stress, increased muscle work during exercise leads to reactive oxygen species (ROS) production. Microbiota might play a role in ROS eradication by different mechanisms. *Lactobacillus, Lactococcus* and *Bifidobacterium genera* were reported to increase glutathione, which helps in ROS neutralization. *Lactobacillus plantarum, Lactobacillus gasseri, Lactobacillus fermentum, Lactococcus lactis* increase the efficiency of Super Oxide Dismutase (SOD). Murine model studies revealed that mice with microflora more abundant in *Escherichia coli* and *Enterococci* while poor in *Lactobacillus* species exhibited increased ROS than those rich in *Lactobacillus*. [19]

ROLE IN POST- EXERCISE REGENERATION AND FATIGUE PREVENTION

Human research on intestinal microflora's role in muscle regeneration and fatigue perception is limited and mainly concerns prebiotic (and probiotic) intake. Post-exercise recovery might be decreased by antiinflammatory properties of the microbiome described in further paragraph.

Petersen et al. noticed an increase in *Prevotella* in highly fit cyclists. *Prevotella* is responsible for branched-chain amino acids (BCAA) synthesis. BCAA, such as leucine, isoleucine and valine are linked to decreased fatigue and increased muscle protein synthesis, which helps in post-exercise recovery. BCAA are mainly exogenous, derived from supplementation, thus an endogenous source would be an optimal solution for improving recovery. Additionally, an increase in *Methanobrevibacter smithi*i was noticed, which might increase intestinal fermentation to molecular hydrogen (H2) [14].

A pilot study conducted by Nobuhiko Eda et al. examined the role of molecular hydrogen (H2) produced by intestinal microbiota on muscle regeneration and fatigue prevention after intense exercise. H2 possesses antioxidant properties that decrease reactive oxygen species (ROS), which cause lipid and protein peroxidation, resulting in muscle damage and dysfunction. This delays recovery and contributes to inflammation. Participants were given H2-producing milk or a placebo before performing intense exercise for 60 minutes. The results revealed that participants who ingested H2-producing milk exhibited lower muscle soreness and fatigue the next day after treatment in comparison to the placebo group. [15]

Gut microflora might also decrease post-exercise fatigue with fibre fermentation. Hadžic et al. in their study gave basketball players insoluble fibre during four weeks of training. While performance didn't change, players reported significantly less fatigue compared to the placebo group. The most probable cause of decreased exertion is SCFAs production, but deeper research is required. [16]

THE MICROBIOTA GUT-BRAIN AXIS ON PHYSICAL PERFORMANCE

Intestinal microbiota have a bidirectional relationship with the brain through, so called, gut-brain axis. Gut-brain axis connects enteric nervous system (ENS) with central nervous system (CNS) through metabolites and neurotransmitters synthesized by microflora. These include SCFAs, serotonin and dopamine, which achieve brain through blood or vagal transmission. The axis regulates cognition, mood, motivation and psychological health, all of which are crucial elements needed to increase sport performance [20]. Striatum is a brain structure responsible for motivation, which occurs through dopamine binding with receptors.

Exercise increases striatal dopamine. Murial models revealed that mice with depleted intestinal microflora exhibited lower striatal dopamine post-exercise. This might be a result of microflora activating spinal afferent neurons and downregulating striatal monoaminooxidase (MAO), which degrades dopamine. [21]

Another way microflora might influence CNS is through SCFAs. Studies show that exercise correlates with increase in production of SCFAs, mainly aforementioned butyrate, which may positively impact brain function. Butyrate crosses the blood-brain-barrier (BBB) and by expressing anti-inflammatory properties it might relieve neuroinflammation caused by endotoxemia originating from increased intestinal permeability. Butyrate increases brain-derived neurotrophic factor, which might possess antidepressant activity and play a role in neurogenesis. This might open up a way for exercise as a method for depression treatment. [22]

Athletes engaged in endurance and resistance training may require up to twice the amount of protein compared to the general population to support protein synthesis, energy production, and gut health. Approximately 10% of protein is not digested and may undergo proteolytic fermentation by bacterial proteases in the colon. Consuming excess protein can result in the production of proteolytic metabolites that exceed the body's capacity to detoxify them, potentially disrupting intestinal barrier function and causing inflammation. To date, only one study has explored the effects of protein supplements on the gut microbiota in athletes (male runners). The results showed that protein powder consumption led to a decrease in the abundance of *Lachnospiraceae, Roseburia, Blautia, Synergistales, Coprococcus, Lactobacillales, Bacilli*, and *Bifidobacterium longum*, as well as an increase in *Bacteroidetes* and a reduction in *Firmicutes* compared to the placebo. There were no significant differences between groups at baseline or after the intervention in terms of α-diversity, β-diversity, or microbial metabolites (e.g., SCFAs, ammonia). While protein supplementation altered the abundance of specific microbial taxa, it had limited effects on overall diversity and microbial function. A protein-rich diet seems to have minimal impact on gut microbiota composition but may lead to an increased production of proteolytic metabolites. [23]

Studies suggest that a high fat diet increases the relative abundance of gut bacterial taxa known for their lipid metabolism capabilities. In their investigation, Burke et al. have investigated the impact of a high fat diet on gut's microbiome: an increase in abundances of Dorea and several OTUs within Bacteroides was revealed. The growth of *Enterobacteriaceae* was also enhanced by the high fat diet. A significant decrease in *Faecalibacterium* and *Bifidobacterium* abundances was observed. [24]

There are numerous studies investigating the influence of high fiber diet on richness and diversity of the gut microbiome. The researchers of those independent studies have shown that the populations whose diet contains higher amounts of fiber exhibit an increased amount of *Prevotella, Actinobatceria* and *Bacteroidetes* compared to those who consume lesser amounts of fibre. High fibre nutrition is linked to decreased abundance of *Ruminococcus, Bifidobacterium, Blautia, Faecalibacterium, Bacteroides, Alistipes* and *Bilophila*, according to the above studies. [25]

INFLUENCE OF PROBIOTICS AND PREBIOTICS ON INTESTINAL MICROFLORA AND ATHLETE'S PERFORMANCE

In their systematic review, Kristensen at al. investigated the impact of probiotics on fecal microbiota composition in healthy adults: the influence of probiotics on gut microbiome composition in healthy adults has not been proven. In most of the studies included, probiotic supplementation had no impact on evenness, richness or α-diversity of fecal microbiome in comparison to the placebo group. However, one study by Ferrario et al. revealed significant alteration in compositional differences (β-diversity) after probiotic deployment: an increase of *Proteobacteria* and *Clostridiales genus Coprococcus*, as well as a decrease in *Clostridiales genus Blautia* in the probiotic group compared to placebo. In this study, α-diversity did not differ in placebo and probiotic groups. [26]

In another study, bodybuilders who consumed a specific high-protein, low-carbohydrate diet were either assigned to the probiotic supplementation group or the placebo group: no positive effect of probiotics on the gut microbiome composition was revealed. [27]

The balance of humans' gut microbiome can be supported by prebiotic supplementation. Prebiotics do not contain bacteria, they include substances (usually plant oligosaccharides) that cannot be degraded by human enzymes; however, they undergo fermentation which is linked to the gut microbiome activity. As a result, short chain fatty acids are produced e. g. acetate, propionate and butyrate. The supplementation of prebiotics aids the proliferation of *Lactobacillus* and *Bifidobacterium,* which prevent the growth of adverse bacterial species. [28]

Prebiotics and fiber may also favor the growth of particular bacterial species indirectly: The prebiotic fermentation performed by one bacterial species gives particular products, which later can be used as substrate for growth by other bacterial species in that community. This phenomenon is called cross-feeding. The studies have revealed, that after deployment of fructans, bifidobacteria and lactobacilli utilize those fructans and as a result, they produce lactate and acetate which then are probably used as a substrate to acquire energy by various bacteria e. g. *Roseburia, Faecalibacterium* and *Eubacterium.*

On the other hand, some fermentation products such as SCFAs and lactic acid decrease the colonic pH which also alters the microbial composition in the intestines: reduced intestinal pH favors the growth of *Firmicutes eg. Roseburia spp.* (which produce butyrate) whereas the growth of *Bacteroides spp*. is suppressed. [29]

Probiotic bacteria enhance the host's ability to maintain microbial balance in the intestines: probiotics prevent the growth of pathogenic intestinal bacteria e.g. *Clostridium perfringes, Salmonella enteritidis*. The probiotic bacteria may also inhibit the production or inactivate other microorganisms' toxins, this prevents food poisoning. The mechanism of action of those probiotics includes adhesion to the epithelial cells, competition with pathogenic microorganisms for nourishment and production of antibacterial substances. The probiotic bacteria adhere to the epithelium and as a result they prevent the colonization of the intestinal wall by the pathogenic bacteria. The metabolism of the probiotic bacteria results in production of antibiotics (acidophiline, bacitracin, lactacin) and low-molecular-weight substances (SCFAs and hydroperoxide), the latter being able to prevent the growth of pathogenic bacteria. The probiotic components also produce high-molecular-weight substances. Probiotic *Lactobacillus* is able to bind iron hydroxide and thus deprives other bacteria of this life-sustaining cation [30]

The impact of probiotics on sports performance has not been widely investigated, the few studies conducted on this topic show farraginous results: most of those investigations reveal no relationship between probiotic supplementation and increasing athletes' performance. However, there are several studies which reveal the rise in ability of aerobic exercise, enhancement of training load and prolongation of time to exhaustion as a consequence of multi-strain probiotics application. An increase in volume of oxygen utilization was also perceived in those studies. A positive impact of single-strain probiotics on aerobic exercise was revealed only in one study so far. [31]

Prebiotics are widely used by athletes to support nutrition. Protein hydrolysates aid skeletal muscles. Plant-derived polyphenols and flavonoids alter the gut microbiome and enhance intestinal barrier which leads to the improvement of athlete's energy level. By modifying gut's microbial composition, oligosaccharides and polysaccharides boost physical performance. A study by Camilla J Williams et al. has revealed, that Oligofructose (FOS)-Enriched Inulin modifies the guts microbiome, which potentially leads to the enhancement of ventilatory threshold, however in the investigation FOS -enriched inulin did not increase maximal oxygen uptake. In another study β-glucan supplementation led to a rise in *Lactobacillus* and *Bifidobacterium* abundance, improvement of handgrip strength and reduction of injury risk. Moreover, supplementation of carbohydrates during physical activity is proven to lengthen the time to fatigue. Citrulline can alleviate visceral hypoperfusion during intensive exercise which prevents the deterioration of physical performance. [32]

INFLUENCE OF DIETARY SUPPLEMENTATIONS ON INTESTINAL MICROBIOTA AND ATHLETE'S PERFORMANCE

Supplementation of protein, probiotics and polyphenols (antioxidants) is strongly proven to interact with intestinal microbiota. Antioxidants are known for their anti-inflammatory role. Plant-derived polyphenols prevent infections and alter the gut's microbiome: flavonoids aid the growth of intestinal *Lactobacilli*, *Bifidobacteria*, *Faecalibacterium prausnitzii*, *Akkermansia* and *Roseburia* which all are perceived as beneficial for human health. [33]

Branched-chain amino acids supplementation leads to a decrease in abundances of *Bifidobacterium dentium* (known for its ability to support mucus layer of the intestines) and *Lacticaseibacillus paracasei* (widely used as a probiotic), however it also caused an increase in *Ruminococcus*. [34]

Next to beneficial traits, dietary supplements such as L-carnitine might also have a detrimental influence on some aspects of human health: L-carnitine is metabolized to trimethylamine (TMA) by the gut's microbiome. Followingly, TMA is transformed into trimethylamine oxide (TMAO) by monooxygenases in the liver. Elevated circulating TMAO levels are linked to higher risk of metabolic and cardiovascular diseases. High concentrations of TMAO form a reflection of intestinal dysbiosis. [35]

The athlete may be more prone to illness in case of macro- or micronutrients deficiency. Thus, dietary supplements may be crucial for enhancing athlete's health and more indirectly- their physical performance. Micronutrients such as vitamins are indispensable for athletes' health: vitamin D impacts innate immunity and may play an important role in treatment and prevention of upper respiratory diseases; vitamin C and zinc also help to alleviate the symptoms, however too high doses of antioxidants such as vitamin E and C might diminish the training induced adaptations. Zinc deficiency is common in athletes and leads to deterioration of the immune system (e.g. lymphoid atrophy). Vitamin C decreases cortisol and interleukin-6 responses to physical activity. [36]

Vitamin supplementation can alter the microbial composition of the intestines and minimize the inflammation of the digestive system of athletes. Many vitamin groups exhibit anti-inflammatory action on the host's organism. Vitamin D can downregulate the expression of inflammatory cytokines e.g. TNF-alpha and IL-6, however the vitamin is also known for its ability to increase the production of antimicrobial substances. Moreover, Vitamin D aids the immune system of the intestines by boosting phagocytosis and raising differentiation of lymphocytes into anti-inflammatory Th2 lymphocytes. Supplementation of vit. D leads to augmentation of the number of lymphocytes CD8+, an increase in intestinal *Bacterioidates* and a decrease in *Proteobacteria* abundances. Vitamins A and E diminish the production of reactive oxygen species and play an antioxidative role in human organism. [37]

CHANGES OF INTESTINAL MICROFLORA IN RESPONSE TO INTENSIVE TRAINING AND PHYSICAL ACTIVITY

Gut microflora diversity seems to be influenced by exercise. Acute and chronic exercise was revealed to increase alpha diversity of gut bacteria, which correlated with increase in SCFAs. These changes reverted after halting exercise, which suggests that microflora needs constant stimulus for the shift to remain. Prolonged, excessive exercise however, might be harmful to the gastrointestinal tract via hypoperfusion and decreased intestinal permeability, which might not be supportive of microflora differentiation. [38]

Engaging in regular physical activity leads to increase in Firmicutes phylum, mainly in *Ruminococcaceae* (*Ruminococcus gauvreaui* and *Faecalibacterium prausnitzii*), *Lachnospiraceae* (*Dorea, Coprococcus, Roseburia*) and *Erysipelotrichaceae* families. High-intensity sports promote increase in *Bifidobacterium* and *Bacteroidetes* species, such as *Prevotella intermedia* and *Bacteroides caccae*. *Proteobacteria phylum* (such as *Escherichia coli*), associated with poor microbiome health, was reported to decrease with moderate exercise, however high-intense sports correlated with increase of the phylum. Increases in *Firmicutes* and *Bacteroidetes* are responsible for greater SCFAs production in athletes. [39,40] *Firmicutes* phylum is mainly responsible for butyrate production, while *Bacteroidetes* mostly generate propionate and acetate. SCFA, especially butyrate, have an impact on pathogenic species. Butyrate through Hypoxia-induced factor (HIF) promotes stabilization of hypoxia in the gut and increases secretion of antimicrobial peptides (RegIIIγ and β-defensins) by the colon cells. Increased SCFA in athletes may play a role in decreasing infection risk. [40]

Numerous studies were done to examine effects of different sports on gut microflora. Yuxue Yi et al. performed a multi-cohort study across three types of sport. Male subjects were divided into training wrestling and aerobics, while female subjects trained rowing or aerobics. Rowers had distinct microbiota suited to high-energy requirements, while wrestlers showed markers of anaerobic performance. Microbiota variability was also influenced by sex, especially with exercise intensity, showing that microflora composition is based on many individual factors. [41]

Not all sports might lead to changes in intestinal microflora. A study by Bycura et al. investigated the influence of resistance exercise and cardiorespiratory exercise on differentiations of microbiota. Fecal samples of subjects engaged in cardiorespiratory exercise showed shifts in gut microflora, especially noticeable in second and third weeks of intervention. Resistance training however, where subjects performed bench press, squats and bent-over barbell rows, didn’t significantly change microbiota at any point. This might point to aerobic physical activity having more impact on the composition of intestinal microflora than anaerobic physical activity, such as resistance training. [42]

SUMMARY

The intestinal microflora, which consists of a variety of microorganisms, is a significant part of the human body, responsible for maintaining the homeostasis and taking part in metabolic processes. The gut microbiota's diversity is dynamic, with each individual hosting a unique composition shaped by factors such as genotype, the initial colonization at birth, and dietary habits. [1, 2] Athletes' intestinal microbiota differs from that of sedentary individuals, reflecting adaptations to intense training. [11] Microorganisms influence the body's inflammatory and regenerative processes through various mechanisms (including through the brain-gut axis), which has a positive impact on endurance and physical performance in athletes. [20, 21] The use of probiotics and prebiotics has also been proven to have a beneficial role in preventing performance deterioration during physical performance. [32]

Exercise, especially intense physical performance (aerobic and anaerobic) in athletes, appears to influence gut microbiota diversity by increasing specific bacterial families. Investigating these changes can help reduce risks of exhaustion, inflammation and resulting from these factors injuries while practicing sports. [41]

Author’s contribution: Conceptualization: NK; methodology: WF, KP, ZM; check: NK; investigation: DGD, ZM, ; resources: WF, KP; data curation: NK, WF, KP, ZM; writing – rough preparation: WF, KP, ZM; writing–review and editing: NK, DGD; visualization: NK supervision: NK, DGD ; project administration: DGD;

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