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Prevalence of *Gardnerella Vaginalis* among pregnant women at the Cité-Verte District Hospital (Central Region – Cameroon)

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Abstract*—Bacterial vaginosis is a genital condition resulting from a profound imbalance in the vaginal ecosystem, with Gardnerella vaginalis as the main agent involved. Scientific data suggests that bacterial vaginosis increases the risk of premature rupture of membranes, infection of amniotic fluid and membranes, preterm labor, and premature delivery. This study aimed at evaluating the prevalence of Gardnerella vaginalis in pregnant women at the Cité-Verte District Hospital. A prospective cross-sectional study with analytical purposes was carried out over three months on pregnant women who came to undergo cytobacteriological examinations. Two vaginal samples were collected from each woman using sterile swabs and tested according to Amsel and Nugent culture criteria. Data on potential risk factors was also collected. Among the 272 pregnant women registered in this study, 124 women (45.58%) were affected by Gardnerella vaginalis. This infection rate was statistically higher among pregnant women aged 20 to 25, with a primary education level, employed and in school, single, having sexual intercourse 5 to 7 times a week, in the second trimester of pregnancy and practicing intimate hygiene with soap. The Bassa, Mbamoise and Beti ethnic groups were the most infected. We demonstrated that Gardnerella vaginalis was associated with yeasts. The results obtained as part of this work show that pregnant women are more exposed to Gardnerella vaginalis in view of their physiological state and external factors. It should also be noted that raising awareness is an effective preventive measure in the fight against bacterial vaginosis*.

***Keywords*—** *Bacterial vaginosis, prevalence, Gardnerella vaginalis, pregnant women, Cité-Verte District Hospital*.

I. INTRODUCTION

Bacterial vaginosis is a pathology due to an imbalance of the vaginal flora with multiple causes (1). Most often, it manifests clinically by vaginal discharges with bad odors and up to 50% of women presenting these symptoms are affected (1). Bacterial vaginosis is characterized as a polymicrobial syndrome resulting from the abnormal proliferation of a variety of anaerobic bacteria, such as *Gardnerella vaginalis* (*G. vaginalis),* which is the main germ involved (2). Other bacteria, such as *Prevotella, Bacteroides* and *Mobiluncus,* and aerobic bacteria such as *Mycoplasma* and *Ureaplasma sp.,* are also responsible for bacterial vaginosis (3). In addition, some data support its possible role in cases of associated infections favoring sexually transmitted diseases (4).

In today's world, it is the most common female genital infection in women of childbearing age and it is estimated that it affects between 5 and 70% of women (5). It is important to note that this condition is most common in some regions of Africa and least common in Asia and Europe (5).

Bacterial vaginosis is more common in sexually active women. This vaginosis can be symptomatic or asymptomatic (6). Genital infections have become a huge health problem in Cameroon with devastating consequences "premature deaths, family destabilization, increase in the number of orphans, increase in medical and funeral expenses, increase in absenteeism at work, decrease in productivity and massive job losses, reduction of skilled labor force, increase in misery and poverty are very blatant" (7).

The increase in vaginal infections in women in general and pregnant women in particular requires special attention not only from health professionals but also from public authorities. Pregnant women constitute a weak and exposed link because during pregnancy there is an increase in oestrogen levels, which leads to an increase in glycogen and other substances deposited in the vagina, thus favoring the development of infection (8).

The consensus on the treatment of bacterial vaginosis is not established because, rather than "eradicating" the infection, some would propose to "restore" the normal vaginal flora by means of lactobacilli (2). Current strategies for combating bacterial vaginosis are limited to prevention.

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Studies on this subject in our society are rare because they require a relatively long follow-up with regular visits. Studies on bacterial vaginosis during pregnancy have been conducted in several countries (9,10,11), but data on prevalence and risk factors associated with pregnant women are insufficient in Cameroon. In addition, epidemiological data on vaginosis due to *Gardnerella vaginalis* in pregnant women, particularly in the city of Yaoundé, are insufficient. It is in this context that this work was developed to evaluate the prevalence of *Gardnerella vaginalis* in pregnant women consulting at the Cité-Verte District Hospital in order to alert the health authorities about the growth of infections due to this bacterium so that it strengthens awareness and prevention strategies against this scourge.

II. MATERIAL AND METHODS

Study Design, Location and Period

A prospective transversal and analytical study was carried out at the Cité-Verte District Hospital (CVDH) in the city of Yaoundé (Central Cameroon region) over a period of 3 months from April 8 to July 10, 2022. The culture, identification of strains, and additional biological analyses were carried out at the CVDH laboratory.

Selection Criteria

All pregnant women who had been prescribed a cervical-vaginal self-sampling by the doctor and had complied with the following conditions were included in our study:

- No personal hygiene in the morning of sampling;
- No sexual relations the day before;
- Acceptance to freely participate in the study.

All pregnant women who did not meet the above conditions were excluded from the study.

Data collection and processing

The data collection medium was a questionnaire sent to each pregnant woman included in our study. A file was created for each patient and contained the following information: age, sex, marital status, religion, level of education, weekly frequency of sexual intercourse, socio-professional status, and knowledge regarding the prevention and complications of vaginal infections. A patient code was generated for greater confidentiality and written on each file.

The Prevalence (P) was calculate using the formula (11):

$$P = \frac{Number\ of\ individuals\ infected}{Number\ of\ individuals\ examined} \text{ X } 100$$

Biological analyzes

Patient sampling

The sample was taken from the 272 pregnant women included in this work. The swabs were labeled with the patient's information necessary for the analysis (name, first name, age, sex, date of collection, nature of collection, and site of collection). When the cervix was visible, the exo-cervix was taken, then the wall using a sterile swab (12). We proceeded directly with a thin spread on the object slide, then the swab was introduced into the swab holder and a few drops of physiological water were added. During the sampling, the appearance of the cervix, the color of the leucorrhea, the consistency, the quantity, and the odor were noted.

Biochemical examinations

- pH measurement: An acidic pH is a diagnostic criterion for bacterial vaginosis (13). After collecting the vaginal secretions using a swab, a part was covered with pH paper, and migration and changes in coloring were observed. The intensity of the coloring being proportional to the acidity of the vaginal environment was compared to the color scale found on the box, which assigned a number corresponding to the pH.
- Potash test or sniff test: this test consists of adding a drop of 10% potash to the sample spread on a slide. This potash lyses the cell bodies and thus gives off a rotten fish odor, which is very suggestive of the combined presence of anaerobic bacteria and *Gardnerella vaginalis* (14). This impractical test was carried out directly at the sampling site.

Microscopic examinations

- Fresh state

The microscopic examinations that were finally carried out are as follows: fresh on a slide with observation using a 10X and 40X objective.

- Gram staining

After making the smear and staining with Gentian violet and Fuchsin, the reading has been done under a microscope using a 100X immersion objective. Gram+ bacteria were colored purple, while Gram- bacteria appeared pink.

The cytology was also noted, i.e., the presence of epithelial cells, leukocytes, which are not specific to the infection, yeasts, and parasites. The presence of indicator cells, “clue cells,” which are coccobacilli lining the gram-variable epithelial cells.

The appearance of the vaginal bacterial flora is typed according to Spiegel (1983), (15).

Type I: Exclusive presence of Doderlein bacilli.

Type II: The Doderlein flora is present and is in the majority, but there is a replacement flora without dominant morphology.

Type III: presence of Doderlein bacilli but predominance of other bacteria (Gram+ and Gram-).

Type IV: absence of Doderlein bacilli and presence of mono- or poly-bacterial flora (Gram+ or Gram-).

We also specified the other germs present in cases of polyinfection. The cocci appear in the form of shells (spherical) of purple color in small chains, long chains, or isolated, and the bacilli (long rods) are purple or pink, respectively, for Gram-positive cocci, Gram-positive bacillus, and Gram-negative bacillus.

Identification of Gardnerella vaginalis

A current international consensus establishes the diagnosis of *Gardnerella* when three of the following criteria are met (16):

- Malodorous and adherent leucorrhoea.
- Vaginal pH greater than 4 or 5
- Positive test of potash
- Presence of clue cells in microscopic examination (17).

Statistical Analysis

The various data collected were recorded in the software Epi-Info version 7.0 and then analyzed using Sotfware SPSS version 22.0 and Microsoft Excel 2019. Quantitative variables

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were presented as mean and standard deviation, and qualitative variables as headcount and percentages. The threshold of statistical significance was set at 5%.

Ethical considerations

During the study, respect for those surveyed and confidentiality of the results were guaranteed. All respondents were informed of the aim and objectives of the study through an oral message before administering the questionnaire. We obtained authorization to conduct an investigation from the director of the Cité-Verte District Hospital before proceeding with data collection. Respect for the anonymity of those surveyed is guaranteed. Additionally, each person gave consent before answering the questions. Participation in the study was therefore voluntary.

III. RESULTS

272 pregnant women were sampled for PCV at the Cité-Verte District Hospital during the period of this study. The average age of our patients was 26 to 39 years old; the youngest was 15 years old, and the oldest was 44 years old.

It appears from Table I that, among the 272 pregnant women sampled, 124 were affected by *Gardnerella vaginalis* infection, with a prevalence of 45.58% of the population studied. That said, 148 pregnant women, or a percentage of 54.41%, were not infected.

Distribution of infection according to age groups

The age group most represented in this study is that of 25–30 years (P = 35.30%), followed by that of 20–25 years (P = 28.7%). We noticed that the prevalence of infection was higher among subjects aged 20–25 (P = 60.25%). Subjects in this age group are also the most infected in the overall study (47 infected subjects out of the 124 total subjects).

Distribution of infection according to education level

According to the results recorded, the prevalence of infection is higher among subjects with primary and secondary education, with prevalence equal to 57.53% and 48.333%, respectively. Those with the lowest infection rate are subjects with a university level (P = 33.33%).

Distribution of infection according to socio-professional status, marital status and frequency of sexual intercourse per week

The prevalence of infections is higher among employed women (P = 54.10%), while those who are unemployed have a low rate of infections (P = 36.49%).

Single women were those whose prevalence of infection was high (P = 53.85%), while this rate is lower among married women (P = 40.79%).

According to the frequency of sexual intercourse per week, women who have sex 5 to 7 times have a high prevalence of infection (P = 67.57%), while those who have sex rarely have a low infection rate (P = 30.95%).

Distribution of infection according Gestational age

In the population of 124 women infected during pregnancy, women in the second trimester had a higher prevalence of infection (P = 74.31%), while those in the third trimester had the lowest rate (P = 31.14%).

Distribution of infection according to Ethnic Groups

The Bassa community, followed by the communities of Mbamoise and Beti, had the highest prevalence (P equal to 66.66%, 57.14%, and 56.82%, respectively). The most represented community is that of the Bamilékés (43.38%).

Distribution of infection according to knowledge of preventive measures and complications of vaginal infections

The prevalence of infection was higher among women who had no knowledge of preventive measures or complications of vaginal infections (61.62%).

Distribution of infection according to the use of vaginal cleansing and the way of vaginal cleansing

Women who practice personal hygiene with soap (60.67%) had a higher prevalence of infection. However, women using simple water and an antiseptic have a lower rate of 38.64% and 37.26%, respectively.

The majority of women do their intimate hygiene externally (68.38%), and the prevalence of *G. vaginalis* is high among women performing internal intimate hygiene (58.14%), also called vaginal douching.

TABLE 1. Distribution of infection in the population according socio-demographic factors.

Groups	Effectives (%)	Prevalence (%)	P value
Age (years)			**0,009094**
15-20	30 (11,02)	11 (36,6)	
20-25	78 (28,7)	47 (60,25)	
25-30	96 (35,30)	42 (43,75)	
30-35	44 (16,17)	16 (36,3)	
35-40	18 (6,6)	6 (33,3)	
40-45	6 (2,2)	2 (33,3)	
Level of study			**0,003313**
No education	60 (22,05)	29 (48,33)	
Primary	59 (16,17)	34 (57,63)	
Secondary	79 (33,82)	36 (45,57)	
Higher	74 (27,94)	25 (33,33)	
Socio-professional status			**0,002335**
Students	76 (27,94)	31 (40,79)	
Employee	122 (44,85)	66 (54,10)	
Unemployed	74 (27,21)	27 (36,49)	
Marital status			0,09982
Maried	152 (55,88)	54 (35,53)	
Single	120 (44,12)	70 (58,33)	
Frequency of sexual intercourse per week			**0,01983**
5 à 7 times	37 (13,60)	25 (67,57)	
3 à 4 times	101 (37,13)	55 (54,45)	
1 à 2 times	92 (33,82)	31 (33,70)	
Rarely	42 (15,44)	13 (30,95)	
Gestational age			**0,00695**
1st trimester	102 (37,5)	62 (60,78)	
2nd trimester	109 (40,07)	81 (74,31)	
3rd trimester	61 (22,43)	19 (31,14)	
Ethnic Groups			**0,03879**
Beti	88 (32,35)	50 (56,82)	
Bamiléké	118 (43,38)	44 (37,29)	
Bassa	24 (8,82)	16 (66,66)	
Mbamoise	14 (5,14)	8 (57,14)	
Foulbé	10 (3,68)	0 (0,00)	
Maka	4 (1,47)	0 (0,00)	

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Peuls	4 (1,47)	0 (0,00)	
Tika	4 (1,47)	4 (100)	
Nanga	4 (1,47)	2 (50)	
Mafa	2 (0,73)	0 (0,00)	
Knowledge of preventive measures and complications of vaginal infections.			0,16105
Yes	186 (68,38)	71 (38,17)	
No	86 (31,62)	53 (61,62)	
Product used for vaginal cleansing			**0,04482**
Plain water	132 (48,53)	51 (38,64)	
Water with soap	89 (32,72)	54 (60,67)	
Water with antiseptic	51 (18,75)	19 (37,26)	
The way of vaginal cleansing			0,15101
Outside	186 (68,38)	74 (39,79)	
Vaginal douching	86 (31,62)	50 (58,14)	
Total	**272**	**124**	

Distribution according to isolated germs associated with G. vaginalis

We obtained, in the context of associated infections, a high frequency of yeasts of around 45.16% (Table 2).

TABLE 2. Distribution according to isolated germs associated with *G. vaginalis.*

Genres	Number (%)
***G. vaginallis* + levure + Gram-positive cocci**	12 (9,67)
***G. vaginallis* + levure**	56 (45,16)
***G. vaginallis* + Gram-positive cocci**	22 (17,74)
***G. vaginallis* + Gram-positive Bacillus**	24 (19,35)
G. vaginallis	10 (8,06)
Total	124 (100)

IV. Discussion

Vaginal infections are common medical problems in women and are associated with substantial discomfort. The objective of our study was to determine the prevalence of pregnant women affected by *G. vaginalis* at the Cité-Verte District Hospital. From April 8 to July 10, 2022, 272 pregnant women were sampled for cervical-vaginal self-sampling at the Cité-Verte District Hospital. These women were recruited on the basis of inclusion and exclusion criteria through consecutive and non-exhaustive sampling. The average age of our patients is 26 to 39 years old; the youngest was 15 years old, and the oldest was 44 years old.

This study revealed that, of the 272 pregnant women sampled, 124 were affected by *G. vaginalis* infection, representing a prevalence rate of 45.58% of the population studied.

This prevalence is similar to that obtained by Kamga et al. in a study conducted in Kumba Health District in Cameroon (P = 55%) (18). This prevalence is also close to that of a study carried out in Sub-Saharan Africa by Govender et al. (19), where the *G. vaginalis* infection rate was between 21 and 52% among pregnant women attending prenatal consultations, and also in another study conducted in Khartoum-Sudan (49.8%) (20). These prevalences could be attributed to socio-demographic characteristics, sexual activity, information on reproductive health, behavioral and genital hygiene of the participants, study design, methods used to search for germs, as well as a temporal and spatial variation in the risk factors for these genital infections (21, 22).

The prevalence of *G. vaginalis* was high among women in the age group of 20–25 years (60.25%), followed by the age group of 25–30 years (43.75%). One study on the prevalence of bacterial vaginosis, conducted in the CDC central clinic in Tiko, Cameroon, shows similar results. This study investigated that bacterial vaginosis was more prevalent in the age group of 20–25 years (48.1%) followed by 25–29 years (44.4%), and the age factor shows a significant association with bacterial vaginosis (23). A study conducted by Konadu in Ghana in 2015 showed the prevalence of bacterial vaginosis, trichomoniasis, and candidiasis among pregnant women (24). This report revealed that 50.55% of the bacterial vaginosis-positive pregnant women were in the age group of 21–30 years, followed by less than 20 years of age, with a prevalence of 29.67% (25). Another study that occurred in other countries in Africa also showed similar results in Senegal (26). Based on this observation, we estimate that this age group corresponds to the period of full sexual activity in women and will influence the flora and vaginal hygiene (25). During the active phase of sexual activity, the composition of the vaginal flora changes and becomes a stratified squamous epithelium rich in glycogen; the pH is acid (4–4.5), and lactobacilli predominate (27). Then the vaginal flora becomes favorable to infection.

The infection rate of *G. vaginalis* is higher in subjects with a primary level of education (57.63%) than in those with no level of education (48.33%). Kamga et al. (18) demonstrated that the prevalence of bacterial vaginosis infection was higher among pregnant women with no level of education (33.3%) (18). The absence of any notion of genital hygiene and poorly managed sexual activity could be an explanation. Ojieabu et al. (28) have previously shown the negative impact of a low level of education on the knowledge and attitudes of Nigerian women about genital infections (28). Most women with no level of education have reading and comprehension difficulties, making it difficult to obtain information on the notions of protection and hygiene.

According to the results obtained, the prevalence of infection is higher among employed women (54.1%) and those in school (pupils and students) (40.79%). Studies conducted in the USA, Poland, China, Indonesia, and Nigeria revealed an association between bacterial vaginosis and different sociodemographic characteristics, such as occupational status (29, 30, 14). This might be due to the variation in prevalence of genital infection in the community, which could be attributed to factors such as hygiene practices and mainly sociodemographic characteristics. Working women and also students are required during their day to use public toilets to relieve themselves and use genital cleaning methods that are not very hygienic compared to those who remain at home; therefore, they are more exposed (31-33).

According to the results of this study, single women are those whose infection rate is high, i.e., 58.33%. These results are similar to those obtained by Dieye et al. (34) in a study conducted in Dakar, Senegal (34). Likewise, Ranjit et al. (35) reported a higher prevalence of bacterial vaginosis in unmarried women (35). On the other hand, studies carried out

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by Koanga et al. (18) and Kamga et al. (22), respectively, in Douala and Kumba, two cities in Cameroon, revealed a high prevalence of bacterial vaginosis among married women (18, 22). Likewise, it should be noted that in the study conducted by Koanga, it was also shown that several women with more than one sexual partner in the last six months were single women (22). This would therefore explain the high infection rate in this group of subjects. According to the study by Dieye et al. (34), single women were at greater risk of contracting a genital infection because they were more likely to have multiple sexual partners (34). This hypothesis seems to be confirmed by the data from this study.

The prevalence of the infection rate increases with the number of sexual relationships. Women who have had sex 5 to 7 times have a high rate of infection (67.57%). The frequency of sexual intercourse promotes the proliferation of germs in the vaginal flora because, at the end of unprotected intercourse, the sperm with a basic pH of 7-9 will disrupt the flora, which has an acidic pH. Then the vaginal flora becomes favorable to infection (27).

In the population of 124 women infected during pregnancy, women in the second trimester had a higher infection rate of 74.31%. Similar to the report of Ibrahim et al. (29) but contradicts the findings of Awoniyi et al. (36) whose prevalence decreased with an increase in trimester. The physiological state of the woman influences the onset of the syndrome. During pregnancy, the pituitary gland continues to stimulate the secretion of estrogen through follicle-stimulating hormone (FSE) and progesterone through luteinizing hormone (LH). Progesterone secreted in large quantities during this period, despite its multiple roles, has an immunosuppressive effect and will modify local immunity. Estrogen promotes the proliferation or even filamentation of yeast, which leads to a reduction in the production of interleukins by the epithelial cells of the vagina. Yeasts secrete proteases, which induce the implantation of germs and their proliferation in the environment, leading to bacterial vaginosis. This result confirms that presented by Schoesman et al. (37) during the study of vaginosis in pregnant women (37). In fact, they demonstrated that the increase in the frequency of bacterial vaginosis would be linked to the mechanism of hormonal impregnation, which influences the microbial population of the vaginal flora.

According to the results, the infection rate is highest in the Bassa community (66.66%), followed by the communities of Mbamoise (57.14%) and Beti (56.82%). The most represented community is that of the Bamilékés (43.38%). This makes us believe that cultures influence vaginal hygiene. An in-depth study within these different communities would be necessary to explain these results.

The prevalence of infection is higher among women who have no knowledge of preventive measures and complications of vaginal infections (61.62%). Thus, an effective fight against genital infections necessarily requires the incorporation of educational campaigns on genital infections into control programs in order to improve the level of knowledge about these infections (28, 38).

Among women who practice personal hygiene with soap, 60.67% were infected by *G. vaginalis*. However, women using simple water and an antiseptic have a lower rate of 38.64% and 37.26%, respectively. Some authors have implicated toiletries as risk factors for vaginosis (39, 40). This remark is similar to that of Fonck et al. (39), who showed that vaginal douching in general and vaginal douching with soap and water in particular were significantly associated with bacterial vaginosis. Some of these products could have antiseptic properties, and others, such as soaps, could greatly disrupt the vaginal environment and thus increase the risk of infection (39). Soap is composed of potassium hydroxide (KO), whose pH is basic, while the vagina has an acidic pH (22, 39). So regular use of soap could contribute to the more or less profound destabilization of the vaginal flora.

The infection rate was high among women practicing internal vaginal cleansing, also called vaginal douche (58.14%), compared to 39.79% among women who perform external vaginal cleansing. These results coincide with a previous study in the same setting that delineated that the incidence of pelvic inflammatory disease like bacterial vaginosis was 45% of douching performers compared to 22% of non-performers (42). A meta-analysis of published studies from 1965 to 1995 did prove that vaginal douching increased the risk for pelvic inflammatory disease by 73% (38). This has been further confirmed by a study conducted in 2005 by Ness and colleagues (43). These results let the Center for Disease Control and Prevention put an obligation on commercial douche boxes to contain warnings about the association between vaginal douching and pelvic inflammatory disease.

In the context of associated infections, we obtained a large association between *G. vaginalis* and yeasts of the order of 45.16%; *G. vaginalis* with infection alone is just 8.06%, which represents the lowest percentage. In other words, in our study, the *G. vaginalis* infection observed is mainly associated with other pathogens. These results were similar to those obtained by the study conducted by Abalaka et al. (44), which found that the coinfection of G. vaginalis with yeasts occurred more frequently compared to that of other germs (44). Moreover, several studies showed a common association between the yeast *Candida albicans*; this is the case of Shey Nsagha et al. (45) and Vincent Khan Payne (11). This could justify the fact that *G. vaginalis* and *C. albicans* were not located in the same area. *G. vaginalis* is usually located at the level of the vaginal normal flora, whereas *C. albicans* is generally found at the level of the vaginal wall, and they also do not use the same resources (11).

V. Conclusion

The aim of this study was to evaluate the prevalence of *Gardnerella vaginalis* among pregnant women at the Cité-Verte District Hospital (Central Region, Cameroon). The high prevalence of *G. vaginalis* obtained shows it could be a health concern for pregnant women receiving care at health Centres. Bacterial vaginosis prevalence was affected by some hygiene behaviors, socio-demographic characteristics, and clinical characteristics. There is a need for comprehensive educational health programs aimed at reducing *G. vaginalis*

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prevalence and guiding the planning and resource allocation of decision-makers for future interventions and research.

Conflicts of interests

The authors declare that there is no conflict of interest.

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Author's contributions

TFC carried out the research work, designed and supervised laboratory work, and revised the manuscript. FEG conceived the study, prepared the first draft of the manuscript, and participated in the revision and formatting of the final version. ESMC: collected data and laboratory work. DANK guided the laboratory work and revised the manuscript. MGSR participated in laboratory work. TYLR, TDA, AAJP, and GAA conceived the study and revised the final manuscript. All authors have read, revised, and approved the final version of the manuscript.

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