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Assessing the Prevalence of Bacterial Vaginosis among Infertile Women in Thi- Qar Province, Iraq

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Authors
Sarmad Qassim Mohammad, *MSc*[1]
Abdullah Khazal Mohsen, *MSc*[2]
Idriss Saleh Jalil, *MSc*[1]
Osama Akram Mohsein, *MSc*[3,4]*

[1] Technical Institute-Baqubah, Middle Technical University, Diyala, Iraq
[2] Al-Muqdad College of Education, Diyala University, Diyala, Iraq
[3] Department of Medical Laboratory Techniques, Mazaya University College, Thi-Qar, Iraq
[4] Thi-Qar Health Directorate, Al Habbobi Teaching Hospital, Thi-Qar, Iraq Thi-Qar Health Directorate, Al Habbobi Teaching Hospital, Thi-Qar, Iraq

*** *Correspondence***
[1]Department of Medical Laboratory Techniques, Mazaya University College, Thi-Qar, Iraq
[2] Thi-Qar Health Directorate, Al Habbobi Teaching Hospital, Thi-Qar, Iraq.
E-mail: osamaakram889@gmail.com

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ABSTRACT

Background: This study aimed to elucidate the predominant bacterial species implicated in the etiology of vaginosis and to determine the most effective antimicrobial agents for their therapeutic management in Iraq.
Materials & Methods: Swab samples were collected through speculum-assisted vaginal examination. The process involved inserting a swab into the vaginal canal and rotating it for at least eight seconds before pulling it out. Standard procedures including microscopy, culture, and antibiotic sensitivity testing were applied to analyze vaginal swab samples.
Findings: *Staphylococcus aureus* was identified as the most common Gram-positive bacterium involved in bacterial vaginosis (44.3 %). *Serratia* species, were not detected at all (0.0 %). Among the Gram-negative bacteria, *Klebsiella* was the most prevalent bacterium (at 18.8%), followed by *Escherichia coli* at (18.1 %). Candida was detected in 9.4% of cases. In antibiotic susceptibility testing, S. aureus exhibited maximum resistance to imipenem (100%) and high susceptibilitye to penicillin, vancomycin, and clindamycin. Gentamicin was the most effective antibiotic against *E. coli* and *Klebsiella spp*, while vancomycin was highly effective against Candida. These results, indicate that the treatment should be directed towards the antibiotics to which the bacteria show the highest sensitivity to achieve optimal therapeutic outcomes.
Conclusion: *S. aureus* was identified as the most common vaginal pathogen in Thi-Qar province, followed by *Klebsiella spp*., and *E. coli*, respectively. These findings provide valuable insights into the prevalence of different bacterial species and the most effective medications. The results will be invaluable for future prospective investigations.

Keywords: Vaginosis, Gram-negative bacteria, Gram-positive bacteria, Antimicrobial resistance, Sexually transmitted diseases.

CITATION LINKS

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Introduction

The vaginal microbiota of healthy women of reproductive age is typically composed of aerobic, facultative anaerobic, and obligate anaerobic bacteria. In most women, *lactobacilli* form a large portion of the vaginal flora [1-2]. Vaginal microbiota maintains a healthy balance by producing lactic acid, and antibiotic compounds, and stimulating the immune system at a basal level to inhibit the growth of harmful bacteria [3]. An imbalance in the type and number of lactobacilli could lead to increased susceptibility to sexually transmitted infections (STIs) and upper genital tract infections by allowing the growth of bacterial pathogens and anaerobic bacteria[4]. Bacterial vaginosis is a common bacterial infection that affects the lower part of the vagina, affecting 29% of women of reproductive age in the United States [5]. A study in Qom revealed that the number of fully identified Gram-positive bacteria involved in BV was much higher than that of fully identified Gram -negative bacteria, and it was found that 70.34% of women who were unable to conceive had bacterial vaginosis. The most prevalent vaginal pathogens were *Staphylococcus aureus* (57.33%) and *Escherichia coli (E coli)* (25.33%), penicillin and gentamicin were the most effective antibiotics against *S. aureus* [7]. Common bacteria associated with BV include *Gardnerella vaginalis*, *Megasphaera spp*., *Atopobium vaginae*, *Dialister spp*., *Mobiluncus spp*., *Sneathia amnii*, *S. sanguinegens*, *Porphyromonas spp*., and *Prevotella spp* [6- 8]. While BV often presents no symptoms, women with BV are more prone to vaginal odor, irritation, and discharge compared to those without BV [9]. Serious health risks linked to BV include increased risk of infertility, adverse pregnancy outcomes, STIs such as *chlamydia*, *gonorrhea*, human papillomavirus (HPV), and human immunodeficiency virus (HIV) infections, along with pelvic inflammatory diseases (PID) like endometritis [10]. It is estimated that 20–30 % of women with vaginal discharge have BV, although the prevalence could reach 50–60 % in some populations with high-risk sexual behaviors [11]. PID and endometritis are upper vaginal infections that could present with various clinical symptoms and signs. Acute (PID) occurs when stringent or facultative anaerobic bacteria move from the vagina to the endometrium and adnexa within a 30-day timeframe [12]. Chronic endometritis is a persistent infection lasting 30 days or more. Bacteria associated with BV and/or STIs are responsible for more than 85% of PID cases. Less than half of PID cases are attributed to *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. The presence of anaerobic bacteria, linked to BV and other diseases, such as *Mycoplasma genitalium*, plays a significant role in the development of endometritis and PID. Both endometritis and PID are linked to numerous adverse health outcomes, including chronic discomfort, ectopic pregnancy, tubo-ovarian abscess, and infertility [13-14]. The vagina typically harbors indigenous *lactobacilli* that make up the normal vaginal microbiota. BV is associated with a decrease in *lactobacilli*, which are usually the dominant bacteria, and an increase in other anaerobes forming a polymicrobial biofilm on the vaginal epithelium [15]. BV is the most common cause of vaginal discharge worldwide but, is asymptomatic in at least 50% of cases. A large body of research suggests that the harmful impacts of BV extend beyond the lower genital tract. BV is closely linked to reproductive issues, including late fetal loss and preterm birth [16]. Multiple studies have demonstrated a high occurrence of BV in individuals with infertility. However, the specific challenges that infertil patients with BV face in terms of pregnancy outcomes have not been definitively determined [17]. Studies also face several challenges, including antibiotic resistance in bacteria due to genetic mutations and changes that occur in bacterial strains, which complicates the antibiotic sensitivity process and impacts the effectiveness of the antibiotics used. In addition, issues related to the study design, sample, and statistical methods used present further challenges.

Objectives: The goal of thise study was to identify the most common causes of BV responsible for infertility in women and to determineing the most effective antibiotics to treat these specific pathogens in clinical settings. In this study, vaginal samples of women with vaginitis were collected and analyzed by cultured and microscopy specific groups of bacteria were identified, and their frequency and drug sensitivity were determined.

Materials and Methods

Study design: In this study, a total of 150 high vaginal swab (HVS) samples were collected from women in the women's wards of Al-Haboubi Teaching Hospital in Al-Nasiriyah city (center of Thi-Qar province in southern Iraq) from January to October 2024 . The patients' age ranged from 25 to 35 years. Standard microbiological procedures were employed to examine the collected HVS samples with various bacterial colors.

Sample collection: After obtaining signed written informed consent from the patients, all samples were taken during vaginal examination using a speculum that was moistened with normal saline before use, avoiding the use of antiseptic cream that could adversely affect bacteria and yeasts. Once the speculum was placed into the vaginal mucosa the swab was inserted into the vaginal opening, rotated for 8 seconds, and then removed. The vaginal swabs were then subjected to traditional microbiological analysis, including microscopy, culture, and antibiotic susceptibility testing.

Isolation and identification: Using conventional microbiological techniques, the presence of yeast, *S. aureus*, *Enterococcus*, and microaerobic and aerobic bacteria was detected in the specimens collected. BV was diagnosed using Gram-stained smear technique. Mueller-Hinton agar, Eosin-methylene blue (EMB) agar, and blood agar plates were streaked to cultivate the samples. The plates were then incubated at 37-°C in an oxygen-controlled chamber for 2 days. While some plates showed growth features of the isolates, many plates showed no growth. The cultures were purified through sub-culturing and stored in a refrigerator for further research. Bacteria collected from vaginitis were identified under aerobic conditions using Gram -staining and conventional biochemical tests such as catalase, oxidase, Mmannitol salt agar (MSA), and hemolysis tests. Hemolytic effects on human blood agar media helped identify *Streptococcus* and *Enterococcus* species. Various methods were used to identify Gram-negative bacteria, including Gram staining that appears red, culture on different media, and biochemical tests like oxidase, catalase, carbohydrate fermentation, and analytical profile Iindex (API) tests.

Antimicrobial susceptibility testing: Each colony was cultured overnight in nutrient broth to assess the isolates' sensitivity to antibiotics. After immersing the sterile swab in the bacterial solution, it was streaked across the Mueller-Hinton agar medium. to ensure even bacterial distribution. Antibiotic discs containing ampicillin, ceftriaxone, imipenem, cephazolin, gentamicin, nitrofurantoin, penicillin, streptomycin, vancomycin, clindamycin, erythromycin, and oxacillin were placed on the agar plates with precise spacing. In addition, the efficacy of various antifungal discs placed on Mueller Hinton agar plates was evaluated against Candida. In this study, all antibiotic discs used were from Liofilchem Co. (Liofilchem, Inc., Italy). Sensitivity analyses were performed according to CLSI standards issued in 2023 using standard strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 29213) as controls to validate the test results.

Ethical approval: The Declaration of Helsinki ethical guidelines for medical research involving human subjects were strictly followed in the conduct of the present investigation (2013). Before collecting the samples, all patients included in this study were properly informed about the research objectives and gave their verbal permission. This study was approved by the Publication Ethics Committee at Thi-Qar Health Directorate, Al Habbobi Teaching Hospital, (No: 645 in August 29, 2023).

Statistical analysis: SPSS software version 26 was used for data analysis (SPSS Software, Inc., Chicago, Ill, USA). Chi-square test was used to evaluate variations in prevalence by gender, age group, and geographic location.

Study limitations: The study faces several constraints, including its focus on a limited subset of participants, which may not fully represent the wider population. Self-reported data from participants may introduce bias when assessing symptoms. Factors such as personal hygiene practices, hormonal influences, and prior antibiotic use could alter the study results. There are also challenges associated with long-term monitoring of treatment effectiveness and its impact on vaginal flora.

Findings

Frequency and percentage of Gram-positive bacterial vaginosis in the study group: In this study, *S. aureus* was identified as the most common Gram-positive bacterium involved in bacterial vaginosis and found in 66 cases (44.3%), and *Enterococcus* was present in three cases (2.0%). The results also revealed that the frequency of *Group B Streptococcus* was four cases (2.7%) (Table 1).

Frequency and percent of Gram-negative bacterial *vaginosis* and *Candida* among the study: *E. coli* was identified in 27 (18.1%) samples, *Klebsiella* was identified in 28 (18.8%) samples, *Proteus vulgaris (P. vulgaris)* was identified in three (2.0%) cases, *Pseudomonas aeruginosa (P. aeruginosa)* was identified in four (2.7%), and *Serratia* was not identified at all (0.0%) (Table 2).

Antibiotic susceptibility/resistance of Gram-positive bacteria involved in BV among the study group: antibiotic susceptibility testing results of Gram-positive bacterial isolates were as, follows (Table 3): *S. aureus* showed complete sensitivity (S) to penicillin, gentamicin, and vancomycin. However, it was entirely resistant (R) to imipenem and demonstrated varying degrees of intermediate resistance (I) to ceph-

Table 1) Frequency and percentage of Gram positive bacteria among the study group

Spp.	Frequency	Percentage
Staphylococcus aureus	66	44.3%
Enterococcus	3	2.0%
Group B Streptococcus	4	2.7%

Table 2) Frequency and percentage of Gram-negative bacteria and Candida among the study group**.**

Spp.	Frequency	Percentage
E.coli	27	18.1%
Klebsiella	28	18.8%
Proteus Vulgaris	3	2.0%
Pseudomonas aeruginosa	4	2.7%
Seratia	0	0.0%

alosporins. *Enterococcus* bacteria exhibited complete resistance to ampicillin, penicillin, and oxacillin, with all samples proving impervious. However, partial resistance was shown to ceftriaxone, erythromycin, and vancomycin. *Group B Streptococcus* bacteria displayed complete sensitivity to cefazolin, but revealed resistance to penicillin, erythromycin, ampicillin, and nitrofurantoin. Notably, gentamicin was primarily effective against *S. aureus*, whereas these isolates showed varying levels of resistance to

Table 3) Antibiotic susceptibility/resistance of Gram-positive bacteria

Variable		***S. aureus***	***Enterococcus***	**Group B *Sterptococcus***
Ampicillin	S (No.)	0	0	0
	I (No.)	66	0	0
	R (No.)	0	3	4
Ceftriaxone	S (No.)	66	0	0
	I (No.)	0	2	0
	R (No.)	0	1	4
Imipenem	S (No.)	0	0	0
	I (No.)	0	2	0
	R (No.)	66	1	4
Cefazolin	S (No.)	0	0	4
	I (No.)	26	0	0
	R (No.)	40	3	0
Gentamicin	S (No.)	61	0	0
	I (No.)	0	2	4
	R (No.)	5	1	0
Nitrofurantoin	S (No.)	0	0	0
	I (No.)	29	2	0
	R (No.)	37	1	4
Vancomycin	S (No.)	66	0	0
	I (No.)	0	0	4
	R (No.)	0	3	0
Oxacillin	S (No.)	44	0	0
	I (No.)	22	3	4
	R (No.)	0	0	0
Penicillin	S (No.)	66	0	0
	I (No.)	0	0	0
	R (No.)	0	3	4
Erythromycin	S (No.)	10	0	0
	I (No.)	56	0	0
	R (No.)	0	3	4
Clindamycin	S (No.)	66	0	0
	I (No.)	0	3	4
	R (No.)	0	0	0

S: Sensitivity; I: Intermediate; R: resistance ampicillin, imipenem, and cephalosporins. It is crucial to note that these data emphasize the need to determine suitable treatments based on lab susceptibility testing. This approach avoids the evolution of resistant bacterial strains and ensures the best therapeutic outcomes for patients.

Antibiotic susceptibility/resistance of Gram-negative bacteria and Candida involved in BV among the study group: The current study aimed to determine bacterial sensitivity to a selected group of antibiotics,. The following results were obtained: *E. coli* bacteria showed no complete sensitivity to ampicillin, although one fifth of the samples showed moderate sensitivity.

Table 4) Antibiotic susceptibility/resistance of Gram negative bacteria and *Candida*

Variable		*E.coli*	*Klebsiella*	*P. vulgaris*	*P. aeroginosa*	*Candida*
Ampicillin	S (No.)	0	0	0	4	0
	I (No.)	27	0	0	0	2
	R (No.)	0	28	3	0	12
Ceftriaxone	S (No.)	27	0	0	0	2
	I (No.)	0	0	0	0	0
	R (No.)	0	28	3	4	12
Imipenem	S (No.)	27	0	0	0	0
	I (No.)	0	1	0	0	12
	R (No.)	0	27	3	4	2
Cefazolin	S (No.)	20	0	0	0	0
	I (No.)	7	28	0	0	12
	R (No.)	0	0	3	4	2
Gentamicin	S (No.)	27	1	0	0	14
	I (No.)	0	27	3	4	0
	R (No.)	0	0	0	0	0
Nitrofurantoin	S (No.)	0	0	0	0	0
	I (No.)	0	0	3	4	1
	R (No.)	27	28	0	0	13
Vancomycin	S (No.)	0	1	3	4	14
	I (No.)	22	0	0	0	0
	R (No.)	5	27	0	0	0
Oxacillin	S (No.)	0	0	0	4	2
	I (No.)	21	28	0	0	12
	R (No.)	6	0	3	0	0
Penicillin	S (No.)	0	1	3	0	14
	I (No.)	19	27	0	0	0
	R (No.)	8	0	0	4	0
Erythromycin	S (No.)	0	0	0	0	0
	I (No.)	13	27	3	4	2
	R (No.)	14	1	0	0	12
Clindamycin	S (No.)	0	0	3	0	2
	I (No.)	17	28	0	4	12
	R (No.)	10	0	0	0	0

Both *Klebsiella* and *P.roteus* vulgaris did not exhibit any susceptibility to this antibiotic, while all *P. aeruginosa* isolates were sensitive. The majority of *Candida* isolates showedpresented resistance to ampicillin and ceftriaxone. *E.coli* bacteria demonstrated complete sensitivity to ceftriaxone, while *Klebsiella* and *Proteus* bacteria showed complete resistance, and some sensitivity was found in *Candida* samples. Imipenem was effective against *E. coli*, although most *Klebsiella* samples showed resistance, with moderate susceptibility observed in one sample. Resistance to imipenem was also detected in *P. vulgaris*, *P. aeruginosa*, and *Candida* isolates. Regarding cefazolin, data analysies results showed varying degrees of sensitivity toin *E. coli* isolates, with intermediate resistance in *Klebsiella* and complete resistance in *P. vulgaris*, and *P.seudomonas aeruginosa*.

Resistance to nitrofurantoin was manifested in *E. coli* and Klebsiella, but no sensitivity was observed in *P. vulgaris*, *P. aeruginosa*, or *Candida* isolates. *Klebsiella*, *P. vulgaris*, and *P. aeruginosa* showed partial to complete sensitivity to vancomycin, while *Candida* demonstrated complete sensitivity. *P. aeruginosa* was sensitive to oxacillin, while the remaining samples were moderately sensitive or resistant. Similarly to vancomycin, both *Klebsiella* and *P. vulgaris* demonstrated partial to complete sensitivity to penicillin. None of the bacteria showed high sensitivity to erythromycin and clindamycin, and they were generally resistant.

These results suggest significant variations in bacterial susceptibility and resistance to antibiotics, which should be considered when prescribing them for the treatments offor bacterial and fungal infections. Thus, treatment should be guided by antibiotic susceptibility testing results to achieve optimal therapeutic outcomes.

Discussion

BV is a common infection of the lower vaginal tract in women of reproductive age. It often leads to foul-smelling vaginal discharge, lowers pregnancy success rates, and increases the risk of sexually transmitted illnesses [18]. This study examined the prevalence of BV among women struggling to conceive, underscoring the importance of detecting and treating infectious vaginitis. To identify the most effective treatment for BV, causative bacteria were isolated through sub-culturing and tested for resistance and susceptibility to various. medications. The study found a prevalence rate of 81.20% for BV among infertile women. *S. aureus* was the most dominant vaginal pathogen, accounting for 44.3% of cases, followed by *Klebsiella spp*.(18.8%), *E. coli* (18.1%), and *Candida* at (9.4%). *S. aureus* was highly susceptible to ceftriaxone, vancomycin, penicillin, and clindamycin, indicating that this bacterium has not developed resistance to penicillin in Thi-Qar. *E. coli* was most susceptible to imipenem, cefphazolin, and gentamicin, while Klebsiella displayed high resistance to most drugs. These results align with the results of other studies conducted in Addis Ababa (41.5%) [19], Kenya (43.1%) [20], Nigeria (37%), and Cameroon [20]. (38%), highlighting BV as a significant concern for Ethiopian women throughout their reproductive years [21-24]. The current study findings are also comparable to the findings of previous research in India regarding the prevalence of S. aureus (41.07%). In Addis Ababa, there were 43 and 28 BV cases caused by *E. coli* (41%) and *K. pneumoniae* (30.5%), respectively [23]. Studies in the United States and Belgium have reported unspecified and 42. BV cases reported, respectively [24]. Of the 163 samples of *G. vaginalis*, 37 (22.7% of the total) samples were identified using the Nugent scoring technique in the Cameroon . These findings are lower than those reported in Cameroon (38%) and Gambia (44.4%) but are were consistent with the results of an Italian study (26.62%) [25-27]. Therefore, BV remains a significant global health issue for women, particularly pregnant women or those trying to conceive. While the number of pharmaceutical companies researching and producing new antimicrobial drugs has decreased, the prevalence of microorganisms resistant to multiple drugs has increased dramatically over the past 20 years. Concerns about the spread of antibiotic-resistant bacteria are a top priority for international health agencies, especially in countries with insufficient funding to combat this issue [28-29]. In the current research, the drug susceptibility pattern of aerobic bacteria was identified using a standard culture medium. *S. aureus* demonstrated the highest sensitivity to vancomycin, penicillin, and clindamycin. Fortunately, in Thi-Qar, this bacteriumg has not yet developed resistanceto penicillin. *E. coli* showed the highest susceptibility to imipenem, cefazolin, and gentamicin. *Klebsiella spp*. exhibited high resistance to the majority of drugs. These findings align with the results of other studies conducted in Bahir Dar [30], Gondar [31], Addis Ababa [18], India [32], and Portugal [33]. Gram-negative bacteria possess a more intricate biological structure than Gram-positive bacteria, which creates a barrier affecting permeability and supports the operation of drug efflux pumps. Gram-negative bacteria display more antibiotic resistance than Gram-positive bacteria [34]. Antibiotic-resistant bacteria employ various methods to evade antibiotic effects, such as removing the medicine from the cell, changing the target site, and altering the folic acid production pathway [35]. The future strategy of the World Health Organization (WHO) will prioritize the research and development of novel antibiotics targeting Gram-negative bacteria resistant to lmultiple drugs and broad-spectrum antibiotics [36]. Local health practitioners should apply the latest antibiotics recommended by the WHO. Instead of prescribing broad-spectrum antibiotics, medical professionals should administer narrow-spectrum medications based on the results of antibiotic susceptibility tests. *Lactobacillus* comprises a significant portion of the bacteria in a healthy, stable vaginal environment [37, 38]. S. aureus, an opportunistic pathogen, exists in the vaginal microbiota of multiple hosts. Many complex factors allow *Lactobacillus* to inhibit the growth of *S. aureus*. In cases of BV, *S. aureus* infection disrupts the natural bacterial flora [39]. *C. albicans* is implicated in 80–92% of yeast infections. However, in this investigation, the prevalence of *Candida* infection was lower than anticipated (9.4%). While *Candida* infection accounts for roughly one-third of all vaginitis, cases, our findings are inconsistent with this frequency [40-41]. Bacterial vaginosis (BV) is typically treated with specific antibiotics. To gauge the susceptibility or resistance of the isolated bacteria and yeast several antibiotic discs were used in this study. S. aureus was found to be highly susceptible to penicillin. However, a study in Pakistan reported that *S. aureus* has exhibited developed resistance to penicillin, with just 26.3% of the strains being susceptible to ampicillin. This resistance to penicillin is primarily due to the presence of ß-lactamases. Therefore, combining penicillin with a ß-lactamase inhibitor produces better results [42]. The study faces several constraints, including its focus on a limited subset of participants, which may not fully represent the wider population. Self-reported data from participants may introduce bias when assessing symptoms. Factors such as personal hygiene practices, hormonal influences, and prior antibiotic use could alter the study results. There are also challenges associated with long-term monitoring of treatment effectiveness and its impact on vaginal flora.

Conclusion

The disparity between the frequency of Gram-positive and Gram-negative bacteria involved in BV was substantial. It was found that 81.20% of women who were unable to conceive suffered from BV. *S. aureus* was the most prevalent vaginal pathogen (44.3%), followed by *Klebsiella spp.*(18.8%), *E. coli* (18.1%), and *Candida* (9.4%). *S. aureus* was primarily susceptible to ceftriaxone, vancomycin, penicillin, and clindamycin. *E. coli* was highly susceptible to ceftriaxone, imipenem, cefazolin, and gentamicin. However, *Klebsiella spp*.. showed resistance to many drugs. It is crucial for physicians to stay vigilant and utilize available screening methodologies to rapidly detect and treat BV patients.

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Ethical permissions: The Declaration of Helsinki ethical guidelines for medical research involving human subjects were strictly followed in the conduct of the present investigation (2013). Before collecting the samples, all patients included in this study were properly informed about the research objectives and gave their verbal permission. This study was approved by thee Publication Ethics Committee at Thi-Qar Health Directorate, Al Habbobi Teaching Hospital, (No: 645 in 29/8/2023).

Authors' contributions: SQM: contributed to study design, literature review, and analysis; AKM: contributed toin experimental analysis, samples collection, and obtaining ethical approval; ISJ: contributed to data analysis, experimental analysis, and data collection; OAM: contributed to study design, sample collection, and data analysis. All authors were contributed to the writing, reviewing, and final proofing of the article.

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