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Table Of Contents

Indonesian Journal on Health Science and Medicine
Vol. 3 No. 1 (2026): July
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PCR-Based Identification and Epidemiological Assessment of Entamoeba gingivalis Among Patients With Periodontal Disorders

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Abstract

General Background: *Entamoeba gingivalis* is an oral protozoan frequently found in dental plaque and periodontal pockets and has been reconsidered for its role in oral pathology. **Specific Background:** Recent molecular approaches have enabled improved detection of this organism in patients with gingivitis and periodontitis. **Knowledge Gap:** Despite growing interest, limited data exist comparing conventional microscopy and polymerase chain reaction (PCR) for accurate detection and subtype identification in clinical samples. **Aims:** This study aimed to determine the prevalence of *E. gingivalis* among periodontal disease patients and to compare the diagnostic performance of microscopy and PCR. **Results:** Among 120 patients, 7.5% tested positive for *E. gingivalis*, while 92.5% were negative. PCR confirmed all positive isolates and demonstrated higher sensitivity in detecting low-level infections, with all isolates showing strong genetic similarity to the ST1 subtype. **Novelty:** This study integrates molecular detection and phylogenetic analysis to confirm subtype distribution in a clinical population. **Implications:** PCR is recommended as a reliable diagnostic tool for accurate detection and epidemiological assessment of *E. gingivalis*, supporting improved identification in periodontal disease research and clinical practice.

Highlights:

- Low detection rate of oral protozoan observed in clinical samples
- Molecular method identifies subtype clustering within regional isolates
- Diagnostic comparison reveals higher sensitivity of amplification-based testing

Keywords: Entamoeba Gingivalis, Periodontal Disease, Polymerase Chain Reaction, Molecular Detection, Phylogenetic Analysis

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Introduction

Entamoeba gingivalis is an anaerobic amoeba that inhabits the human oral cavity, particularly within the gingival sulcus, periodontal pockets, and tonsillar crypts. It was first described by Gros in 1849 and is currently classified within the phylum Amoebozoa. Unlike its pathogenic relative Entamoeba histolytica, E. gingivalis has historically been considered a commensal organism with limited clinical relevance. However, recent studies have increasingly questioned this assumption, revealing potential associations between the presence of E. gingivalis and periodontal diseases such as gingivitis and periodontitis [1],[2].

Morphologically, E. gingivalis exists only in the trophozoite form and lacks a cyst stage, indicating that direct contact is necessary for transmission. This includes oral behaviors such as kissing, sharing utensils, or using contaminated dental tools. Its detection is often underestimated due to its fragile structure and the limitations of traditional microscopic techniques. However, advances in molecular diagnostics, particularly polymerase chain reaction (PCR)-based assays, have significantly improved the sensitivity and specificity of E. gingivalis detection in clinical samples [3],[4].

There is growing evidence suggesting that E. gingivalis may not be a mere bystander but rather an active participant in the pathogenesis of periodontal inflammation. Its increased prevalence in individuals with periodontal disease compared to healthy controls supports this hypothesis. Some studies have demonstrated the amoeba's ability to phagocytize host cells, including epithelial cells and leukocytes, and to contribute to tissue destruction through enzymatic degradation and immunomodulation [5],[6].

The renewed interest in E. gingivalis stems not only from its potential pathogenicity but also from the broader understanding of the oral microbiome and its impact on systemic health. As periodontal disease has been linked to systemic conditions such as cardiovascular disease, diabetes mellitus, and adverse pregnancy outcomes, identifying all contributors to periodontal pathology is of critical importance. In this context, E. gingivalis emerges as a potentially underestimated factor requiring further investigation [7].

Recent molecular and microbiome-based studies have emphasized the interaction between E. gingivalis and bacterial communities within periodontal pockets. Evidence suggests that the amoeba may enhance bacterial virulence by transporting pathogenic bacteria intracellularly, thereby contributing to dysbiosis and deep-pocket colonization [8],[9]. This cooperative behavior highlights the need to view periodontal disease as a polymicrobial infection in which protozoa may act synergistically rather than independently [10],[11].

Moreover, emerging research indicates that E. gingivalis may influence host immune responses through modulation of cytokine production and evasion of neutrophil-mediated killing. These immunological alterations may accelerate periodontal tissue degradation and impair healing processes, ultimately exacerbating disease severity [12,13]. Understanding these mechanisms is crucial for the development of targeted diagnostic and therapeutic strategies aimed at reducing protozoan-mediated periodontal damage [10].

Materials and methods

1. Samples collection

A total of 120 patients with periodontal diseases (periodontitis and gingivitis) were enrolled in the current study who attended the Specialized Dental Center /Bab Al- Muadham, Baghdad. Their age ranged between 15 and 65 years, from 7th / August / 2024 to 27th / January/ 2025.

2. Microscopic examination

Samples of dental plaque were collected from each patient by a sterile swab wiped around the teeth and around the gingival crevices, the swabs were immersed in tubes containing normal saline and the swab smeared on the slide, stained with Giemsa stain and examined under a microscope.

3. DNA extraction

Nine samples were selected to be examined by using Polymerase chain reaction (PCR) technique for the detection of 18S-the small subunit of ribosomal DNA gene (SSU rDNA) gene with 200 bp and comparing the results. Entamoeba gingivalis were recognized by their shape depending on the presence of vacuoles and the expansion of the pseudopodia formation (14).

The genomic DNA was extracted from the isolates using a commercial wizard genomic DNA purification kit (Promega, USA). PCR was used for the detection of the 18S-SSU rDNA gene. DNA was extracted from the samples, which were then stored at −20C° until use.

4. Primer design

The primers used were universal primers; a forward primer (5-AGGAATGAACGAACGTACA-3) and a reverse primer (5'-CCATTTCCTTCTTCTATGTTTCAC- 3).

Indonesian Journal on Health Science and Medicine
Vol. 3 No. 1 (2026): July
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5. Singleplex PCR

The thermal cycler conditions were 40 cycles at 94°C 1 min (denaturing), followed by 56°C 2 min (annealing) and 72°C 1 min, and finally 72°C 5 min for final extension. Each PCR reaction was carried out in a final volume of 25 µL. The components of the reaction mixture included 12.5 µL of master mix, 5 µL of DNA sample (100 ng), 2.0 µL of each forward and reverse primer, and 3.5 µL free-nuclease distilled water. PCR products and 200 bp DNA molecular ladder (Promega) the positive result of the 18S-SSU rDNA gene is visualized in by 2% (w/v) agarose gel electrophoresis. Gels were stained with ethidium bromide solution (0.5 µg/mL)(1).

Statistical analysis

The analysis was done by SPSS (Statistical Package for Social Sciences) version 20. For all analysis, statistical significance was considered at highly significant level P-value of <0.01, significant level P-value of <0.05 and non-significant level P-value >0.05.

Ethical issues

All patients were willing to participant and they informed about the data and specimens I collected from them and considering ethics when verbal information collected.

Results

Table (1) Shows the overall distribution of Entamoeba gingivalis infection among the examine specimens. Out of a total of 120 samples, 9 samples (7.5%) tested positive, while 111 samples (92.5%) tested negative. This indicates a low prevalence of positive cases within the tested group

Table (1): Distribution of Positive and Negative Samples

Samples type	Count	Percentage%
Positive samples	9	7.5 %
Negative samples	111	92.5%
Total	120	100%

Table (2) Among the 120 total samples, 86 were from males and 34 from females. Out of the male samples, 8 (6.7%) tested positive while 78 (65.0%) tested negative. For the female group, only 1 sample (0.8%) was positive and 33 (27.5%) were negative. This indicates a higher prevalence of positive cases among males compared to females.

Table (2): Distribution of Positive and Negative Samples according to the sexes

Sexes	Total samples	Positive samples	Negative samples	Positive (%)	Negative (%)
Male	86	8	78	6.7 %	65 %
Female	34	1	33	0.8 %	27.5 %
Total	120	9	111	7.5%	92.5 %
Chi-square		X2 =0.65	P= 0.419	Non-significant	

Table (3) Presents the prevalence of Entamoeba gingivalis infection among patients with gingivitis, categorized by age group. The study involved a total of 120 individuals, and the data is distributed across five age groups. Each group lists the number of total visitors, positive cases and negative cases, along with their corresponding percentages. Although older age groups show a slightly higher prevalence of infection, the association between age and infection with Entamoeba gingivalis is not statistically significant.

Table (3): Distribution of Positive and Negative specimens according age groups

Age group (years)	Total visitors	Positive cases	Percentage (%)	Negative cases	Percentage (%)
15-25	21	0	0%	21	100%
26-35	16	1	6.25%	15	93.75%
36-45	28	1	3.57%	27	96.43%
46-55	25	3	12%	22	88%
56-65	30	4	13.33%	26	86.67%
Total	120	9	7.5%	111	92.5%
Chi-square	X2 =4.56	P=0.335	Non-significant differences		

The table (4) Presents the distribution of various risk factors potentially associated with gingivitis and Entamoeba gingivalis infection among the study participants. The most prevalent risk factors were poor oral hygiene (60%) and lack of regular dental care (55%). Other notable factors included periodontal disease (50%), high sugar intake (35%), and smoking (25%) respectively.

Indonesian Journal on Health Science and Medicine
Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.437

Table (4) Prevalence of Potential Risk Factors Associated with Gingivitis and Entamoeba gingivalis Infection

Risk factors	No. of patients	Percentage (%)
Poor oral Hygiene	72	60.0%
Periodontal Disease	60	50.0%
Smoking	30	25.0%
Immunosuppression	6	5.0%
Dentures/ Orthodontic devices	18	15.0%
Close contact/ Saliva exchange	24	20.0%
Advance age	24	20.0%
Lack of regular Dental care	66	55.0%
High Sugar Intake	42	35.0%
Alcohol Consumption	18	15.0%

Figure (1): Entamoeba gingivalis gene picture obtained by agarose gel electrophoresis (2% agarose, 100 V for 90 minutes). DNA ladder 100-1500 bp molecular weight marker; Lane M marker. Entamoeba gingivalis isolates' molecular detection results revealed that the PCR products in nine lanes were positive, indicating the migration of a DNA band based on molecular weight at 200 bp PCR product sizes, which were visible using ethidium bromide staining.

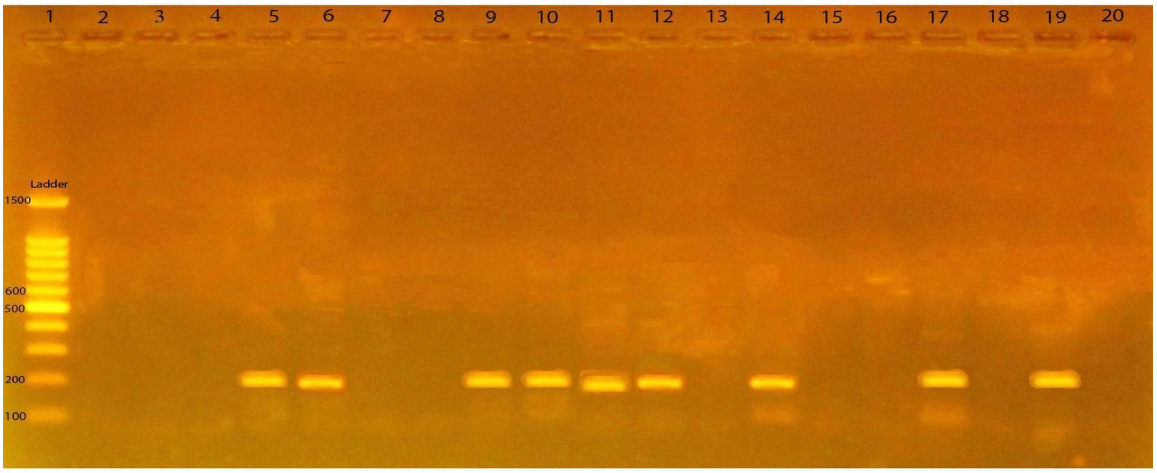
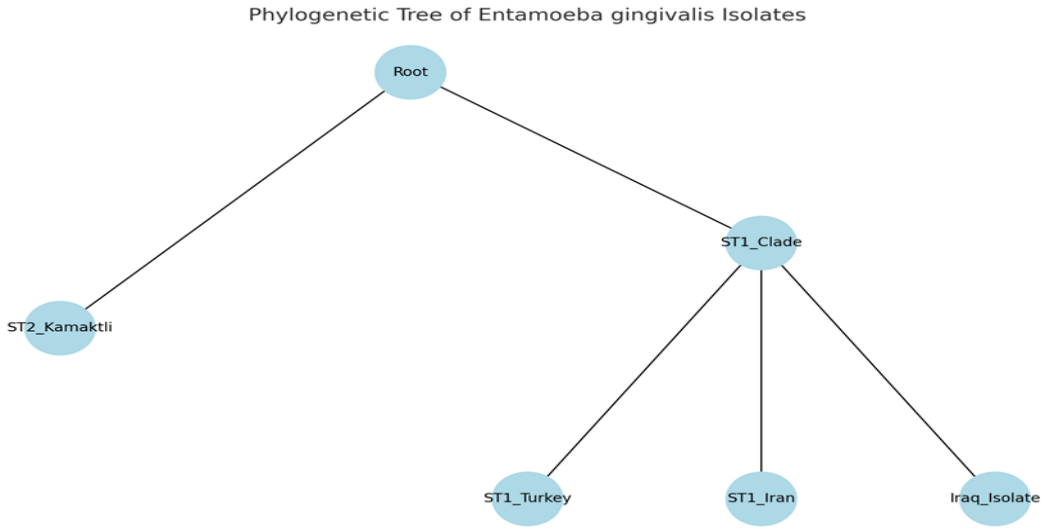


Figure (2): The phylogenetic tree illustrates the genetic relationship between the Iraqi isolate and other isolates from neighboring regions (Turkey and Iran), in addition to the known subtype ST2 "variant". The Iraqi isolate clusters closely with the ST1 clade, which includes isolates from Turkey and Iran. This suggests a high genetic similarity with ST1-type isolates. The ST2 lineage, while divergent, has been reported primarily from outside the region (e.g., Mexico), and no strong evidence links it to the Iraqi isolate.



Discussion

Table (1) of this study presents the overall distribution of Entamoeba gingivalis among the examined samples. The findings suggest a relatively low prevalence of E. gingivalis infection in the studied population. However, this percentage, though

Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.437

numerically limited, remains clinically relevant, particularly when interpreted within the context of oral health and parasitic colonization patterns.

Entamoeba gingivalis is a protozoan parasite commonly residing in the human oral cavity, particularly in gingival crevices and periodontal pockets. It has been frequently observed in association with poor oral hygiene and periodontal disease. While traditionally considered a non-pathogenic commensal organism, recent evidence suggests a more active role in the pathogenesis of gingivitis and periodontitis, especially in susceptible hosts or when oral hygiene is compromised [1],[15].

The low detection rate observed in this study is comparable to findings in certain populations with relatively good oral hygiene or where access to dental care is adequate. For instance, in a study by [16] conducted in Turkey, the prevalence of E. gingivalis was reported to be 11.2%, which aligns with the current study's findings and supports the notion that the parasite is not universally prevalent but rather contextually dependent. In contrast, higher rates up to 80% have been documented in patients with chronic periodontitis or in populations with inadequate oral hygiene practices [15],[17].

Several methodological and population-specific factors may contribute to the relatively low prevalence recorded. First, the diagnostic method plays a crucial role; while microscopic examination is widely used, its sensitivity is limited compared to PCR-based methods. The current study employed PCR, a highly sensitive and specific tool for parasite detection, which likely minimized false negatives. Nonetheless, the low rate suggests a true low-level colonization in the study group rather than a detection bias [3].

Furthermore, the population's demographic and behavioral characteristics, such as age, oral hygiene habits, smoking status, and frequency of dental visits, may have impacted the infection rate. The presence of E. gingivalis is known to correlate with the degree of periodontal pocket depth and inflammation, as demonstrated in the study by [18], where individuals with advanced periodontal disease had a significantly higher presence of the parasite.

The implications of these findings are multifaceted. On one hand, the low prevalence may reflect good oral health standards in the sample group. On the other, it raises important questions regarding the transmission routes, colonization dynamics, and role of host immunity in modulating E. gingivalis infection. Additionally, given that this parasite can be transmitted via saliva and dental instruments, understanding its epidemiology remains essential in the context of dental practice and public health [19].

In conclusion, while the general prevalence of Entamoeba gingivalis found in this study is low, the findings underscore the need for continued surveillance and more in-depth studies to explore its potential pathogenic role in oral diseases. Future research with larger sample sizes and comparative groups (e.g., periodontitis vs. healthy individuals) is warranted to better elucidate the clinical significance of this organism [20].

Table (2) presents the distribution of Entamoeba gingivalis infection based on sexes. Despite this apparent difference in prevalence, the statistical analysis indicates that the association between gender and infection rate is not statistically significant.

The observed pattern of higher infection rates among males is consistent with several prior studies in the field of oral parasitology. Previous investigations have noted a similar gender disparity, often attributing the higher prevalence in men to differences in oral hygiene practices, smoking habits, hormonal influences, and access to routine dental care [1],[17]. Men, in many populations, are less likely to maintain rigorous oral hygiene routines and more likely to engage in behaviors that predispose them to periodontal diseases, which may facilitate colonization by E. gingivalis [21].

In a molecular study by [18], male patients with periodontitis showed a significantly higher detection rate of E. gingivalis compared to females. This finding supports the theory that periodontal pocket depth and inflammation often more pronounced in men can influence the ability of the parasite to colonize and persist in the oral cavity. Additionally, testosterone has been proposed as a modulator of immune response, possibly influencing susceptibility to certain infections, including protozoan colonization [22].

Nonetheless, the current study did not reveal a statistically significant difference between males and females. This could be attributed to the relatively small number of positive cases, particularly among females, which limits the power of statistical inference. It's also worth considering that E. gingivalis transmission may depend more on direct oral contact, salivary exchange, and the presence of predisposing oral conditions rather than on inherent biological differences between sexes [23].

Interestingly, similar trends were noted by [15], who found a non-significant but higher prevalence of E. gingivalis in males within periodontal disease cohorts. In contrast, some studies report no gender difference, suggesting that while behavioral and lifestyle factors may differ, they may not always translate into statistically meaningful differences in infection rates, especially when sample sizes are small [16].

In clinical terms, this gender-based variation, though statistically non-significant in this study is still valuable. It highlights the importance of addressing gender-specific risk behaviors in public health strategies for oral hygiene and periodontal disease prevention. Targeted educational campaigns and regular dental checkups, especially for at-risk groups, may help reduce the overall burden of parasitic colonization and improve oral health outcomes [24].

Table (3) illustrates the distribution of Entamoeba gingivalis infection across different age groups among 120 patients. Although the overall prevalence of infection is low (7.5%), there is a notable trend indicating that the frequency of positive cases increases with age. No infections were observed in the 15–25 age group, while the highest prevalence was found in individuals aged 56–65 years (13.33%). However, the statistical analysis (Chi-square = 4.56, p = 0.335) suggests that the

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Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.437

associate.

This trend of increased E. gingivalis positivity in older individuals is consistent with previous reports suggesting that age is an important risk factor for oral protozoan colonization. As individuals age, the cumulative exposure to periodontal pathogens increases, and the likelihood of chronic periodontal disease, poor oral hygiene, and immune senescence becomes more pronounced [21],[25]. These factors may facilitate the colonization and persistence of opportunistic protozoa.

Research by [1],[15] has demonstrated a higher prevalence of E. gingivalis among patients with chronic periodontal disease, a condition more commonly diagnosed in middle-aged and elderly populations. Moreover, age-related reductions in salivary flow, mucosal immunity, and dental care engagement can further increase vulnerability to oral parasitic infections [17],[23].

While the current study does not report statistically significant age-based differences, the observed trend aligns with biological plausibility and epidemiological patterns seen in other parasitic and periodontal infections. It is important to note that the sample sizes in each age group were relatively small, particularly in younger categories, which may limit the statistical power to detect meaningful associations [26].

Interestingly, the absence of E. gingivalis infection in the youngest group (15–25 years) may reflect better oral health status, more frequent dental visits, or stronger innate immune responses. Conversely, the elevated rates in older adults may underscore the need for targeted screening and preventive strategies in this demographic [25].

From a clinical and public health standpoint, the findings suggest that age-specific oral hygiene interventions and routine periodontal assessments for older individuals may be beneficial in mitigating the risk of parasitic colonization and subsequent periodontal deterioration. Furthermore, integrating molecular diagnostics in age-stratified oral health surveillance could provide more accurate epidemiological data and inform treatment strategies [27].

Table (4) outlines the prevalence of several potential risk factors observed among individuals with gingivitis and Entamoeba gingivalis infection. The most common factors reported in the study include poor oral hygiene, lack of regular dental care, periodontal and gingival disease and high sugar intake. Other less prevalent but still notable risk factors include smoking, advanced age, dentures/orthodontic devices and alcohol consumption.

These results highlight a multifactorial landscape for the development of gingivitis and potential colonization by E. gingivalis. Among the identified risk factors, poor oral hygiene remains the most significant and widely reported contributor to both gingivitis and protozoal colonization. Numerous studies have demonstrated a strong correlation between inadequate oral hygiene practices and an increased risk of harboring E. gingivalis, particularly in individuals with plaque accumulation and gingival inflammation [26,29].

The lack of regular dental care (55%) also appears as a prominent contributing factor. Irregular dental checkups reduce the chances of early detection and treatment of gingival inflammation, allowing pathogenic protozoa to establish and proliferate in periodontal pockets. Research by [17] supports this observation, emphasizing that routine dental visits significantly reduce oral parasite load through mechanical debridement and professional prophylaxis.

Smoking (25%) is another critical factor that has long been associated with increased risk for periodontal diseases and impaired immune response. Cigarette smoke alters the oral microbiome and promotes anaerobic conditions that may favor E. gingivalis survival. In fact, smokers often exhibit deeper periodontal pockets and greater tissue destruction, which may enhance the parasite's ability to colonize sub gingival regions [30].

High sugar intake (35%) can indirectly promote E. gingivalis colonization through its role in increasing bacterial plaque formation and inflammation. Diets rich in refined carbohydrates foster the growth of acidogenic bacteria and increase gingival inflammation, which may compromise mucosal integrity and allow opportunistic protozoa to invade [31].

Less frequent but still relevant factors include dentures/orthodontic devices (15%), which can act as physical reservoirs for microbial and parasitic biofilms if not adequately cleaned. Alcohol consumption (15%) may contribute through its known effects on salivary flow reduction, mucosal barrier disruption, and systemic immune suppression [32],[33].

The multifactorial nature of these findings underscores the complexity of interactions between host behavior, immune function, and microbial ecology in the oral cavity. It also emphasizes the importance of comprehensive patient education, behavior modification, and preventive care in minimizing both the occurrence of gingivitis and the potential for E. gingivalis colonization [34].

The phylogenetic tree presented in Figure (3) demonstrates the genetic relationship of the Iraqi Entamoeba gingivalis isolate in comparison with isolates from neighboring countries (Turkey and Iran), as well as with known subtypes (ST1 and ST2). The Iraqi isolate clusters closely with ST1, which also includes isolates from Turkey and Iran. This indicates a high genetic similarity within regional isolates, likely reflecting shared environmental and epidemiological factors. On the other hand, the ST2 lineage shows a clear divergence from ST1 and has been primarily reported from geographically distant regions such as Mexico and parts of Latin America. This observation supports the hypothesis that ST2 is not widely distributed in the Middle East. Consequently, the present findings suggest that local isolates in Iraq are more closely related to the ST1 lineage, indicating a potential regional epidemiological pattern. These results are consistent with recent studies done by [35] whom demonstrated that isolates from the Middle East predominantly cluster within ST1, with ST2 being rarely detected. And by [36] which showed that the divergence between ST1 and ST2 reflects geographic distribution rather than

Vol. 3 No. 1 (2026): July
DOI: 10.21070/ijhsm.v3i1.437

pathogenicity. further supported that Entamoeba gingivalis isolates from the Middle East exhibit limited genetic diversity compared to isolates from distant regions [37].

Recommendation

Future research with larger sample sizes and comparative groups (e.g., periodontitis vs. healthy individuals) is warranted to better elucidate the clinical significance of this organism

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