

A Novel Integrative Approach to Oral Microbiome Dysbiosis in a Patient with Polycystic Ovary Syndrome Using Pulsatilla 1M: A Pilot Case Report

Jayasree V¹, Sonnymon R², Rachana Mol R S³,
Smera Eldhose⁴, Ramya J K⁵

^{1,2}Department of Organon of Medicine & Centre for Research,
Sarada Krishna Homoeopathic Medical College & Hospital,
Kulasekharam, Kanyakumari District, Tamil Nadu, India.
(Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai)

³Travancore Institute for Bioscience Research

^{4,5}Department of Pathology & Microbiology, Rosy Royal Homoeopathic Medical College & Hospital,
Bangalore, India

Corresponding Author: Jayasree V

E.mail: drjayasreevskhmc@gmail.com

ORCID: <https://orcid.org/0009-0008-6517-8842>

Abstract:

Background: Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder affecting women of reproductive age and is associated with systemic complications such as insulin resistance, obesity, and cardiovascular disease. Emerging evidence suggests that PCOS may also influence the composition of the oral microbiome, potentially compounding oral health problems, yet this relationship remains largely unexplored. A prior systematic review of the impact of PCOS on the oral microbiome demonstrated a significant association between the two, underscoring the role of dysbiosis in both oral and systemic health. This study aims to evaluate the effect of the homoeopathic remedy Pulsatilla 1M on the oral microbiome of a woman diagnosed with PCOS.

Methodology: Oral swabs were collected from the patient before and after administration of Pulsatilla 1M. Samples were serially diluted, plated on nutrient agar, and incubated at 37°C for 24–48 hours. Colony Forming Units (CFUs) were enumerated, isolates were purified by the streak-plate method, and microbial identification was performed using Gram staining and standard biochemical tests (indole, methyl red, Voges–Proskauer, and citrate utilisation).

Results: Pre-intervention cultures showed heavy, confluent growth (10^{-2} dilution: too numerous to count; 10^{-3} dilution: 189 CFU) with a mixed flora of Streptococcus species (Gram-positive cocci) and

Pseudomonas species (Gram-negative bacilli; citrate-positive). Post-intervention, colony counts fell markedly (10^{-2} dilution: 143 CFU; 10^{-3} dilution: 18 CFU) — an approximate 3-log reduction in bacterial load — with only occasional *Streptococcus* colonies detected and *Pseudomonas* species no longer isolated.

Discussion: The reduction in pathogenic colony counts and the disappearance of *Pseudomonas* species following Pulsatilla 1M administration suggest a possible modulatory effect on oral microbial homeostasis, plausibly mediated through systemic hormonal and immune regulation. These preliminary findings offer a new perspective on the potential role of homoeopathy in addressing microbiome alterations linked to endocrine disorders such as PCOS.

Conclusion: This pilot case study provides preliminary evidence that Pulsatilla 1M may help restore oral microbial balance in PCOS, supporting the case for larger, controlled, integrative studies.

Keywords: Polycystic Ovary Syndrome; Oral Microbiome; Pulsatilla; Dysbiosis; Homoeopathy; Pilot Study

1.0. INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorders affecting women of reproductive age. It is characterised by a combination of hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology, with its diagnosis based on established international criteria^[1,2]. Beyond its reproductive manifestations, PCOS is associated with a range of metabolic and long-term health consequences, including insulin resistance, obesity, dyslipidaemia and increased cardiometabolic risk^[1–3]. The pathophysiology of PCOS is complex and multifactorial, involving interactions among hypothalamic–pituitary–ovarian axis dysfunction, insulin resistance, compensatory hyperinsulinaemia, androgen excess and chronic low-grade inflammation^[2,3].

Increasing evidence suggests that alterations in the human microbiome may also be associated with the pathophysiology of PCOS. Early investigations predominantly focused on the gut microbiome, where dysbiosis has been associated with metabolic dysfunction, hyperandrogenism and other reproductive characteristics of PCOS^[4–12]. The proposed relationship between gut microbial dysbiosis and PCOS has been described through mechanisms involving altered intestinal permeability, microbial metabolites, endotoxin exposure, inflammation and metabolic disturbances^[4]. These observations have subsequently stimulated interest in microbial communities at other anatomical sites, including the oral cavity.

1.1. PCOS and the Oral Microbiome

The oral cavity harbours a highly diverse microbial ecosystem that interacts continuously with host tissues and contributes to oral and systemic health. Maintenance of an appropriate microbial equilibrium is important for oral homeostasis, whereas microbial imbalance or dysbiosis has been associated with oral diseases including dental caries and periodontal disease^[22,23]. The oral microbiome is also increasingly being investigated in relation to systemic metabolic and inflammatory disorders.

Emerging evidence indicates that PCOS may be associated with alterations in the oral and salivary microbiome. Lindheim et al. reported differences in salivary microbial composition among women with

PCOS and suggested associations between the salivary microbiome and disease-related parameters ^[13]. Li et al. subsequently demonstrated dysbiosis of the saliva microbiome in women with PCOS using culture-independent microbial profiling ^[14]. Earlier work by Akcalı et al. also identified an association between PCOS, oral microbiota and systemic antibody responses, suggesting a potential interaction between microbial and host immune processes ^[15].

The relationship appears to extend beyond individual microbial taxa. A systematic review examining microbial composition across different body sites reported evidence of microbial alterations in women with PCOS, including changes involving the oral cavity ^[16]. More recently, Huang et al. reported differences in oral–gut microbiome characteristics between women with PCOS and healthy controls, further supporting the possibility that microbial alterations in PCOS may occur across anatomically connected microbial niches ^[17]. However, heterogeneity in study populations, diagnostic criteria, microbiological techniques and potential confounding factors means that the precise clinical significance of these alterations remains uncertain.

The association between PCOS and oral health is further supported by evidence concerning periodontal disease. Proposed mechanisms include hyperandrogenism, insulin resistance, chronic low-grade inflammation and altered host–microbial interactions ^[18–20]. Systematic review and meta-analysis findings have reported an association between periodontal disease and PCOS, although causality and the directionality of this relationship remain incompletely established ^[19,20]. A recent review has also highlighted the potential relationship between oral microbiota and female reproductive health disorders, providing further rationale for investigating the oral cavity in reproductive endocrine disorders ^[21].

1.2. Research Gap

Despite increasing interest in microbiome alterations in PCOS, the oral microbiome remains comparatively less investigated than the gut microbiome. Furthermore, much of the available evidence is observational and primarily describes associations between PCOS and differences in microbial composition ^[13–17]. Whether such microbial alterations can be modified through therapeutic interventions remains insufficiently explored.

A further gap concerns homoeopathic interventions. Published reports have described the use of individualized homoeopathic treatment in PCOS, including preparations such as *Pulsatilla*, with outcomes involving menstrual regularity, clinical symptoms, body mass index and ultrasonographic or hormonal parameters ^[24–28]. For example, previous reports have specifically described *Pulsatilla* in the management of PCOS, while an experimental study published in 2024 investigated *Pulsatilla nigricans* in women with PCOS ^[25,26]. However, these studies have not investigated changes in the cultivable oral bacterial profile as an outcome.

Thus, there remains limited evidence concerning the intersection of PCOS, oral microbial alterations and homoeopathic intervention. Investigating this relationship may provide a preliminary basis for understanding whether changes in the oral microbial environment can be observed following an intervention directed at a systemic endocrine disorder.

2. Rationale and Novelty of the Present Study

The present pilot case study was undertaken to explore changes in the cultivable oral bacterial profile following administration of Pulsatilla 1M in a patient diagnosed with PCOS. Oral swab samples were evaluated before and after intervention using quantitative culture, colony-forming unit (CFU) enumeration, Gram staining and standard biochemical identification.

The study differs from previous PCOS–oral microbiome investigations because it uses a paired pre-intervention and post-intervention assessment in an individual patient, with microbiological evaluation serving as the principal outcome. Importantly, the study does not attempt to characterise the complete oral microbiome; rather, it evaluates changes in cultivable oral bacteria detectable using the selected culture-based methodology.

The study was therefore designed as an exploratory and hypothesis-generating investigation. Any observed changes should not be interpreted as definitive evidence of therapeutic efficacy or restoration of the complete oral microbiome. Instead, the findings may provide preliminary evidence to support the design of larger controlled studies incorporating culture-independent microbiome profiling, periodontal assessment and relevant endocrine, metabolic and inflammatory parameters.

3. Hypothesis

Administration of individualized Homoeopathic treatment with Pulsatilla 1M may be associated with changes in the cultivable oral bacterial profile in a patient with PCOS.

4. Aim and Objectives

Aim

To evaluate the changes in the cultivable oral bacterial profile following administration of Pulsatilla 1M in a patient diagnosed with PCOS.

Objectives

1. To assess the cultivable oral bacterial load before administration of Pulsatilla 1M using colony-forming unit (CFU) enumeration.
2. To identify the predominant cultivable bacterial isolates before and after administration of Pulsatilla 1M using Gram staining and standard biochemical tests.
3. To compare the pre-intervention and post-intervention cultivable oral bacterial profiles following administration of Pulsatilla 1M.

5. Materials and Methods

5.1 Study Design and Setting

This was a single-patient, pre–post interventional pilot case study conducted in a patient clinically diagnosed with Polycystic Ovary Syndrome (PCOS). The study was undertaken at the Sarada Krishna Homoeopathic Medical College & Hospital, Kulasekharam.

The study was designed as an exploratory, hypothesis-generating investigation to assess changes in the patient's cultivable oral bacterial load and detectable bacterial profile following administration of the individualized Homoeopathic remedy Pulsatilla 1M.

5.2 Ethical Considerations

The study was conducted in accordance with the ethical requirements of the institution. Written informed consent was obtained from the patient before enrolment, oral sample collection and administration of the Homoeopathic intervention. The patient's confidentiality and anonymity were maintained throughout the study and during preparation of the manuscript.

5.3 Patient Selection and Intervention

A patient diagnosed with PCOS according to established diagnostic criteria was selected for the pilot investigation. The Homoeopathic remedy was selected on the basis of individualized case assessment and repertorial analysis. Pulsatilla 1M was administered as the prescribed intervention according to the clinical protocol followed by the investigator. Details regarding the patient's clinical characteristics, diagnostic criteria, remedy selection, dosage, administration and follow-up schedule were documented in the case record.

5.4 Oral Sample Collection

Oral swab samples were collected from the patient under aseptic conditions at the predefined pre-intervention and post-intervention time points. The same sampling procedure and sampling site were used at both time points to facilitate paired comparison. The collected swabs were processed under standard microbiological conditions for bacterial isolation and enumeration.

5.5 Microbial Isolation and Enumeration

The collected oral samples were subjected to serial dilution using sterile diluent. Appropriate aliquots from the serial dilutions were inoculated onto nutrient agar plates using standard aseptic techniques. The inoculated plates were incubated at 37°C for 24–48 hours.

Following incubation, bacterial colonies were examined and colony-forming units (CFUs) were enumerated at appropriate dilution levels. Plates showing countable colonies were used for quantitative assessment, while plates showing confluent or too-numerous-to-count growth were recorded accordingly. Representative colonies with distinct morphological characteristics were selected for purification using the streak-plate method. The purified isolates were subsequently subjected to Gram staining and biochemical characterisation.

5.6 Colony Morphology

The isolated colonies were examined macroscopically for characteristics including size, shape, margin, elevation, surface, texture and pigmentation. Representative colony morphology was documented before further identification.

Where confluent growth occurred in the primary inoculation areas, well-isolated colonies obtained following subculture were selected for further examination. Colony morphology alone was considered presumptive and was not used as definitive evidence of species identification.

5.7 Gram Staining and Biochemical Characterisation

Purified bacterial isolates were subjected to Gram staining to determine their Gram reaction and cellular morphology.

Presumptive identification of the isolates was further undertaken using standard biochemical tests, including:

- Indole test
- Methyl Red (MR) test
- Voges–Proskauer (VP) test
- Citrate utilisation test

The biochemical characteristics were interpreted together with Gram-staining results and colony morphology to obtain a presumptive identification of the bacterial isolates.

Because culture-based identification and the selected biochemical tests have limitations for definitive species-level identification, organisms were reported at the species level only where the available phenotypic findings supported such identification. Where definitive identification was not possible, isolates were reported at the appropriate taxonomic level, such as *Streptococcus* species or *Pseudomonas* species.

5.8 Outcome Measures

The primary microbiological outcome was the change in cultivable oral bacterial load, assessed by CFU enumeration before and after administration of Pulsatilla 1M.

Secondary microbiological observations included:

- Change in the detectable bacterial profile.
- Presence or absence of individual bacterial groups.
- Gram reaction and morphological characteristics of the isolates.
- Biochemical characteristics of the isolated organisms.

5.9 Data Analysis

The pre-intervention and post-intervention CFU counts were compared descriptively because the study involved a single patient. Changes in bacterial growth and detectable bacterial groups were documented and interpreted qualitatively and quantitatively where appropriate.

No inferential statistical test was applied because a single-patient design does not provide an adequate sample size for statistical estimation of treatment effects.

6. OBSERVATION & RESULTS

6.1. Colony-Forming Unit (CFU) Assay

A reduction in the cultivable oral bacterial load was observed following administration of Pulsatilla 1M. At baseline, the plate corresponding to the 10^{-2} dilution demonstrated confluent growth and was therefore recorded as too numerous to count (TNTC). At the 10^{-3} dilution, 189 colonies were counted.

Following the intervention, 143 colonies were observed at the 10^{-2} dilution and 18 colonies at the 10^{-3} dilution (Table 1). Based on the calculated CFU/mL values provided for the study, the cultivable bacterial load decreased from approximately 18.9×10^4 CFU/mL before intervention to 1.8×10^3 CFU/mL after intervention.

This represents an approximately 10.5-fold reduction in the calculated cultivable bacterial load, corresponding to approximately a 1- $\log_{10}$ reduction. Because the study involved a single patient and culture-dependent assessment, this finding is presented as a descriptive change rather than evidence of a statistically established treatment effect.

Dilution	Before intervention	After intervention
10^{-2}	TNTC	143
10^{-3}	189	18

Table 1. Colony-forming unit (CFU) counts before and after administration of Pulsatilla 1M.

TNTC: Too numerous to count.

6.2. Colony Morphology

The nutrient agar plates demonstrated dense and confluent bacterial growth in the pre-intervention sample, particularly at the lower dilution, consistent with the high baseline cultivable bacterial load.

Following administration of Pulsatilla 1M, the corresponding plates demonstrated markedly fewer and more discrete colonies across the evaluated dilutions. This visual reduction in colony density was consistent with the decrease observed in CFU counts.

Representative colonies obtained following purification by the streak-plate method were small, circular, smooth and creamy-white in appearance. Colony morphology was recorded as a preliminary characteristic and was considered together with Gram staining and biochemical reactions for subsequent presumptive identification.

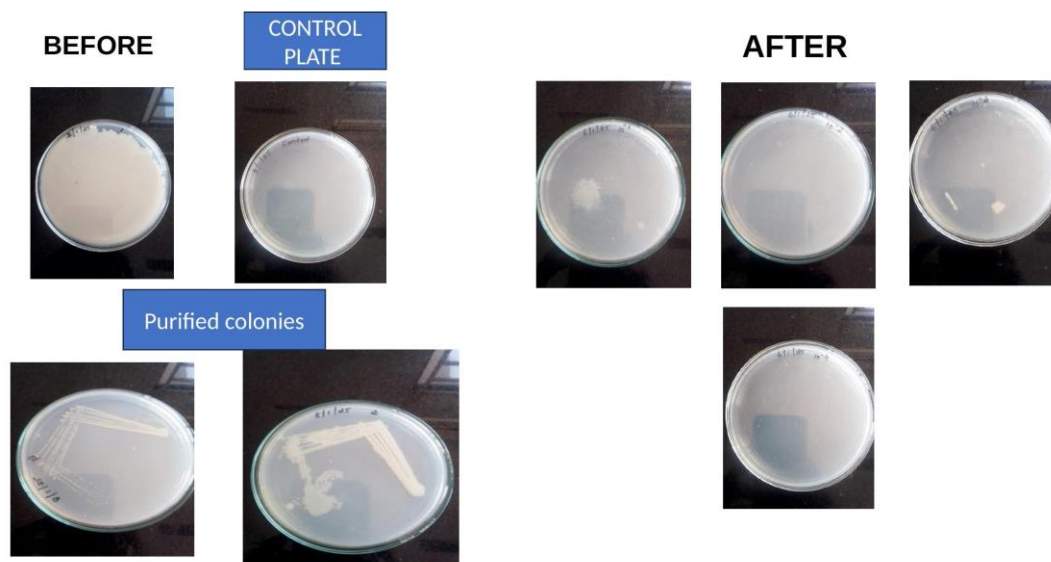


Figure 1. Nutrient agar culture plates showing cultivable bacterial growth in the pre-intervention and post-intervention oral samples following administration of Pulsatilla 1M, together with representative purified bacterial colonies obtained by the streak-plate method.

6.3. Gram Staining

Gram staining of the pre-intervention isolates demonstrated a mixed cultivable bacterial profile consisting of Gram-positive cocci and Gram-negative bacilli. The Gram-positive cocci were predominantly observed in pairs and/or chains, while the Gram-negative organisms appeared as bacillary forms.

Based on the combined Gram-staining characteristics, colony morphology and biochemical reactions, the isolates were presumptively identified as *Streptococcus* sp. and *Pseudomonas* sp., respectively.

Following the intervention, only occasional Gram-positive cocci were detected in the examined preparation. Gram-negative bacilli were not observed in the post-intervention sample under the conditions used for culture and microscopic examination.

Thus, *Pseudomonas* sp. was not detected in the post-intervention culture, whereas occasional *Streptococcus* sp. colonies remained detectable.

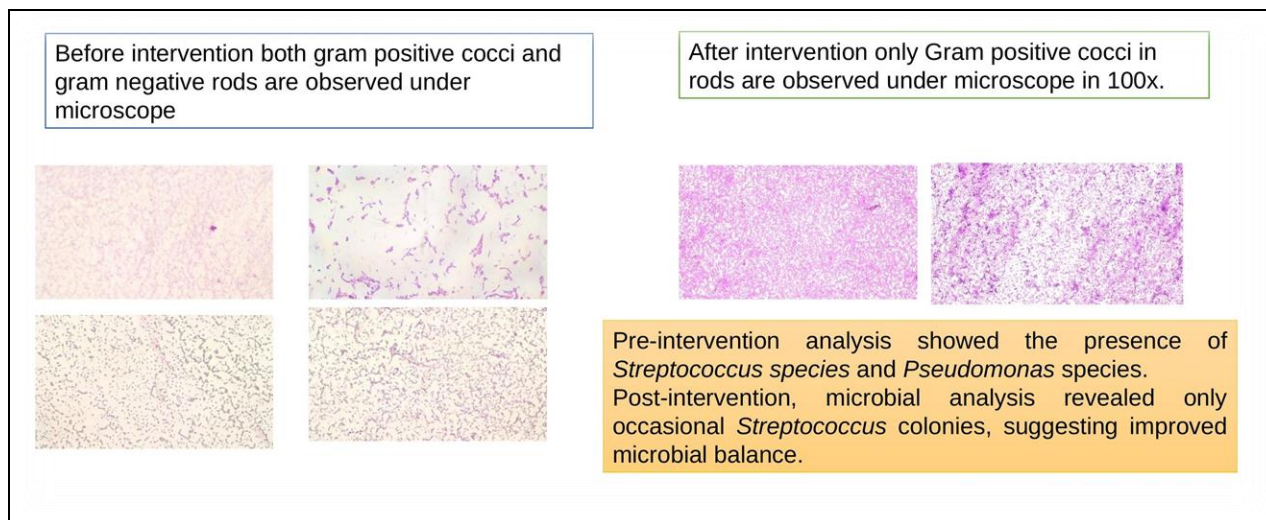


Figure 2. Gram-stained photomicrographs (100 $\times$) of representative oral bacterial isolates before and after administration of Pulsatilla 1M. The pre-intervention sample demonstrates Gram-positive cocci and Gram-negative bacilli, whereas the post-intervention sample demonstrates occasional Gram-positive cocci with no Gram-negative bacilli detected.

6.4 Biochemical Characterisation

The biochemical characteristics of the purified isolates were consistent with their preliminary Gram-staining and morphological profiles.

Both isolates were negative for indole, methyl red and Voges–Proskauer reactions. The isolate provisionally identified as *Pseudomonas* sp. demonstrated positive citrate utilisation, whereas the isolate provisionally identified as *Streptococcus* sp. was citrate-negative (Table 2).

The combined findings from colony morphology, Gram staining and biochemical testing supported the presumptive identification of the isolates as *Pseudomonas* sp. and *Streptococcus* sp. As molecular identification was not performed, the findings should not be interpreted as definitive species-level identification.

Biochemical test	<i>Pseudomonas</i> sp.	<i>Streptococcus</i> sp.
Indole	Negative	Negative
Methyl Red	Negative	Negative
Voges–Proskauer (VP)	Negative	Negative
Citrate utilisation	Positive	Negative

Table 2. Biochemical characteristics of the bacterial isolates obtained from the oral samples.

6.5 Overall Microbiological Findings

The paired microbiological assessment demonstrated three principal changes following administration of Pulsatilla 1M:

- Reduction in cultivable bacterial load: the calculated bacterial concentration decreased from approximately 18.9×10^4 CFU/mL to 1.8×10^3 CFU/mL.
- Reduction in colony density: culture plates showed a transition from dense/confluent growth before intervention to fewer and more discrete colonies after intervention.
- Change in detectable bacterial profile: the pre-intervention sample demonstrated presumptive *Streptococcus* sp. and *Pseudomonas* sp., whereas the post-intervention sample demonstrated only occasional *Streptococcus* sp., with *Pseudomonas* sp. not detected.

These findings represent changes in the cultivable oral bacterial profile and should not be interpreted as comprehensive changes in the entire oral microbiome.

6.6 Summary of Findings

Parameter	Before intervention	After intervention	Observed change
Calculated bacterial load	18.9×10^4 CFU/mL	1.8×10^3 CFU/mL	Marked reduction
10^{-2} dilution	TNTC	143 CFU	Reduced growth
10^{-3} dilution	189 CFU	18 CFU	Reduced growth
Predominant detectable organisms	<i>Streptococcus</i> sp. + <i>Pseudomonas</i> sp.	Occasional <i>Streptococcus</i> sp.	Simplification of detectable profile
<i>Pseudomonas</i> sp.	Detected	Not detected	No longer detected post-intervention
Gram-positive cocci	Detected	Occasionally detected	Reduced
Gram-negative bacilli	Detected	Not detected	No longer detected

7.0. DISCUSSION

The present pilot case study demonstrated a marked reduction in cultivable oral bacterial load following administration of Pulsatilla 1M in a patient with PCOS. The pre-intervention culture demonstrated heavy bacterial growth, with mixed *Streptococcus* and *Pseudomonas* species detected, whereas the post-intervention cultures showed a substantial reduction in CFU counts and only occasional *Streptococcus* colonies. *Pseudomonas* species were not detected after the intervention. These observations indicate a temporal change in the cultivable bacterial profile during the study period.

The magnitude of the observed reduction is noteworthy; however, it should be interpreted within the methodological limitations of the study. Culture-based enumeration detects only microorganisms capable of growing under the selected culture conditions. Therefore, a reduction in CFU cannot be equated with a reduction in total oral microbial diversity or restoration of the complete oral microbiome. Contemporary studies of PCOS and oral microbiota have frequently used 16S rRNA sequencing to characterise microbial communities more comprehensively [13,14,16,17]. Consequently, the present findings are more accurately described as a reduction in cultivable oral bacterial load and alteration in the detectable bacterial profile rather than definitive evidence of microbiome restoration.

7.1. Relationship between PCOS and Oral Microbial Alterations

The observed findings are of interest in the context of emerging evidence linking PCOS with alterations in the oral microbial environment. Salivary microbiome studies have demonstrated differences between women with PCOS and healthy controls ^[13,14], while previous research has also identified associations between PCOS, oral microbiota and systemic antibody responses ^[15]. These findings suggest that endocrine, metabolic and immune abnormalities associated with PCOS may coexist with alterations in oral microbial ecology.

The biological plausibility of such an association is supported by the systemic nature of PCOS. Hyperandrogenism, insulin resistance and low-grade inflammation may influence host–microbial interactions through alterations in metabolic substrates, salivary characteristics and immune responses ^[1–3]. However, these mechanisms were not directly investigated in the present patient. No measurements of insulin resistance, androgen concentrations, inflammatory biomarkers, salivary composition or immune responses were performed in conjunction with the microbiological assessment. Therefore, the present findings cannot establish a mechanistic link between PCOS-related systemic abnormalities and the observed bacterial changes.

The findings should also be considered alongside evidence linking PCOS with periodontal disease. Systematic reviews have reported an association between PCOS and periodontal disease and have proposed inflammatory and metabolic mechanisms as possible contributors ^[19,20]. Nevertheless, the present study did not include periodontal indices, gingival examination, probing depth, clinical attachment level or other measures of periodontal health. Therefore, the disappearance of detectable *Pseudomonas* species cannot be interpreted as evidence of periodontal improvement. It represents a microbiological observation requiring confirmation in future studies.

7.2. Possible Relevance of the Gut–Oral Microbial Axis

The present findings may also be viewed within the broader context of microbiome alterations associated with PCOS. The Dysbiosis of Gut Microbiota (DOGMA) hypothesis proposed that intestinal microbial alterations may contribute to increased intestinal permeability, endotoxin exposure, inflammation, insulin resistance and hyperandrogenism ^[4]. Subsequent studies have demonstrated associations between gut microbial composition and metabolic or reproductive characteristics of PCOS ^[5–12].

More recent research has expanded this concept to multiple microbial sites. Sola-Leyva et al. reported microbial alterations across different body sites in PCOS ^[16], while Huang et al. reported oral–gut microbiome dysbiosis in women with PCOS ^[17]. These findings raise the possibility that oral microbial alterations may form part of a broader systemic host–microbiome interaction.

Nevertheless, the present case does not provide direct evidence for a gut–oral mechanism. Gut microbiota, intestinal permeability, circulating endotoxin concentrations and inflammatory markers were not assessed.

Therefore, the reduction in oral bacterial load cannot be attributed to correction of gut dysbiosis or modification of the gut–oral axis. Such mechanisms remain potential hypotheses for future investigation.

7.3. Pulsatilla 1M and the Observed Microbiological Change

The use of Pulsatilla in PCOS has previously been described in homoeopathic literature. A published case report described the use of individualized homoeopathic treatment involving Pulsatilla in a patient with PCOS ^[24], while Islam and Chowdhury reported a PCOS case treated with individualized homoeopathic prescribing involving Pulsatilla ^[25]. In addition, Gunchala reported an experimental study specifically evaluating *Pulsatilla nigricans* in PCOS ^[26]. A case series of individualized homoeopathic management of PCOS has also reported improvement in clinical and selected laboratory parameters ^[27]. These reports provide a context for investigating Pulsatilla in PCOS, but they do not establish its effect on oral microbial communities.

The present study therefore adds a different outcome domain by examining cultivable oral bacteria before and after administration of Pulsatilla 1M. The temporal association between administration of the remedy and reduction in bacterial counts is an interesting observation. However, because the study involved only one patient and lacked a control group, it is not possible to determine whether Pulsatilla 1M was responsible for the observed microbiological change.

Several alternative explanations remain possible. Oral bacterial populations can fluctuate naturally over time and may be influenced by diet, oral hygiene, salivary flow, recent antimicrobial exposure, menstrual or hormonal changes and other behavioural factors. Regression to the mean may also contribute when the baseline measurement is unusually high. These factors are particularly important when interpreting a single pre–post observation.

7.4. Possible Biological Mechanisms

The mechanism through which an ultra-high-dilution homoeopathic preparation such as Pulsatilla 1M might produce a biological effect remains unresolved. Experimental studies have proposed that certain highly diluted and succussed preparations may contain nanoparticles or exhibit physicochemical characteristics associated with the starting material ^[30,31]. These observations have been proposed as possible explanations for biological activity at extreme dilutions.

However, such hypotheses remain scientifically debated and were not investigated in the present study. No physicochemical analysis of Pulsatilla 1M, nanoparticle assessment, molecular investigation or immunological measurement was undertaken. Therefore, the nanoparticulate hypothesis cannot be used to explain the microbiological findings of this case. It should be regarded only as a proposed theoretical framework requiring independent experimental validation.

Similarly, it would be premature to attribute the observed bacterial reduction to modulation of the hypothalamic–pituitary–ovarian axis, immune function or systemic inflammation. Demonstrating such a mechanism would require simultaneous measurement of endocrine, metabolic, immunological and microbiological parameters.

7.5. Strengths and Limitations

An important strength of this pilot investigation is the use of paired microbiological measurements obtained before and after intervention in the same patient, thereby reducing inter-individual variation. Quantitative CFU assessment was complemented by Gram staining and biochemical tests, allowing a preliminary characterisation of the cultivable bacterial profile.

The principal limitation is the single-patient design. The absence of a control group prevents attribution of the observed change specifically to Pulsatilla 1M. The culture-dependent methodology also provides only a partial representation of the oral microbial community. Furthermore, the study did not assess periodontal status, salivary parameters, inflammatory biomarkers, hormonal concentrations or gut microbial composition. Potential confounding factors such as diet, oral hygiene and other behavioural changes may also have influenced the results.

Accordingly, the present findings should be regarded as preliminary and hypothesis-generating rather than confirmatory evidence of efficacy.

8.0. Implications for Future Research

The findings provide a rationale for further investigation using a larger and methodologically robust design. Future studies should include an adequate sample size, healthy and/or appropriate PCOS comparator groups, repeated microbiological measurements and standardised monitoring of oral hygiene, diet and antimicrobial exposure.

Culture-independent techniques, particularly 16S rRNA sequencing or shotgun metagenomic analysis, would permit more comprehensive assessment of microbial diversity and community structure. Quantitative culture could be retained as a complementary measure of viable cultivable organisms.

Future research should also integrate oral microbiological outcomes with clinical and biochemical measures of PCOS, including androgen status, insulin resistance, inflammatory biomarkers and menstrual characteristics. Standardised periodontal assessment should be included to determine whether microbiological changes are associated with clinically meaningful changes in oral health.

Randomised controlled studies will ultimately be required to determine whether the observed changes are reproducibly associated with Pulsatilla treatment rather than natural microbial variation, regression to the mean or behavioural and contextual factors.

The present pilot case study identified a substantial reduction in cultivable oral bacterial load and a change in the detectable bacterial profile following administration of Pulsatilla 1M in a patient with PCOS. These findings are broadly relevant to the emerging literature linking PCOS with oral microbial alterations^[13-17] and the reported association between PCOS and periodontal disease^[19,20].

9. CONCLUSION:

The findings of the study do not demonstrate restoration of the complete oral microbiome, it establish a causal therapeutic effect, or provide evidence for a specific hormonal, immunological or nanoparticulate mechanism. The principal value of the study is therefore its exploratory and hypothesis-generating nature. It identifies a potentially novel research question concerning the relationship between PCOS, cultivable oral bacteria and homoeopathic intervention and provides a preliminary foundation for larger controlled studies employing comprehensive microbiome analysis and clinically relevant PCOS outcomes.

REFERENCES:

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod.* 2004;19(1):41-47.
2. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Fertil Steril.* 2018;110(3):364-79.
3. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. *Lancet.* 2007;370(9588):685-97.
4. Tremellen K, Pearce K. Dysbiosis of gut microbiota (DOGMA)--a novel theory for the development of polycystic ovarian syndrome. *Med Hypotheses.* 2012;79(1):104-12.
5. Guo Y, Qi Y, Yang X, Zhao L, Wen S, Liu Y, et al. Association between polycystic ovary syndrome and gut microbiota. *PLoS One.* 2016;11(4):e0153196.
6. Kelley ST, Skarra DV, Rivera AJ, Thackray VG. The gut microbiome is altered in a letrozole-induced mouse model of polycystic ovary syndrome. *PLoS One.* 2016;11(1):e0146509.
7. Lindheim L, Bashir M, Münzker J, Trummer C, Zachhuber V, Leber B, et al. Alterations in gut microbiome composition and barrier function are associated with reproductive and metabolic defects in women with polycystic ovary syndrome (PCOS): a pilot study. *PLoS One.* 2017;12(1):e0168390.
8. Liu R, Zhang C, Shi Y, Zhang F, Li L, Wang X, et al. Dysbiosis of gut microbiota associated with clinical parameters in polycystic ovary syndrome. *Front Microbiol.* 2017;8:324.
9. Torres PJ, Siakowska M, Banaszewska B, Pawelczyk L, Duleba AJ, Kelley ST, et al. Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism. *J Clin Endocrinol Metab.* 2018;103(4):1502-11.

10. Liang Y, Ming Q, Liang J, Zhang Y, Zhang H, Shen T. Gut microbiota dysbiosis in polycystic ovary syndrome: association with obesity--a preliminary report. *Can J Physiol Pharmacol.* 2020;98(11):803-9.
11. Mammadova G, Ozkul C, Yilmaz Isikhan S, Acikgoz A, Yildiz BO. Characterization of gut microbiota in polycystic ovary syndrome: findings from a lean population. *Eur J Clin Invest.* 2021;51(4):e13417.
12. Zhou L, Ni Z, Yu J, Cheng W, Cai Z, Yu C. Correlation between fecal metabolomics and gut microbiota in obesity and polycystic ovary syndrome. *Front Endocrinol (Lausanne).* 2020;11:628.
13. Lindheim L, Bashir M, Münzker J, Trummer C, Zachhuber V, Pieber TR, et al. The salivary microbiome in polycystic ovary syndrome (PCOS) and its association with disease-related parameters: a pilot study. *Front Microbiol.* 2016;7:1270.
14. Li N, Li Y, Qian C, Liu Q, Cao W, Ma M, et al. Dysbiosis of the saliva microbiome in patients with polycystic ovary syndrome. *Front Cell Infect Microbiol.* 2021;10:624504.
15. Akcalı A, Bostanci N, Özçaka Ö, Öztürk-Ceyhan B, Gümüş P, Buduneli N, et al. Association between polycystic ovary syndrome, oral microbiota and systemic antibody responses. *PLoS One.* 2014;9(9):e108074.
16. Sola-Leyva A, Martín-Lagos J, Cobo T, et al. Microbial composition across body sites in polycystic ovary syndrome. *Reprod Biomed Online.* 2023.
17. Huang F, Chen J, Zhou M, Tang R, Xu S, Zhao Y, et al. Dysbiosis of the oral-gut microbiome in PCOS patients and its implication for noninvasive diagnosis. *Clin Transl Med.* 2024;14(8):e70001. doi:10.1002/ctm2.70001.
18. Dou Y, Hu X, Li W, et al. Bidirectional association between polycystic ovary syndrome and periodontal disease: mechanisms and evidence. *Front Endocrinol (Lausanne).* 2023.
19. Márquez-Arrico CF, Silvestre-Rangil J, Gutiérrez-Castillo L, Martínez-Herrera M, Silvestre FJ, Rocha M. Association between periodontal diseases and polycystic ovary syndrome: a systematic review. *J Clin Med.* 2020;9(5):1586.
20. Bitonti V, Perri T, Cigni L, Familiari D, Vazzana G, Franco R. The association between periodontal disease and polycystic ovary syndrome: a systematic review and meta-analysis. *Dent J (Basel).* 2025;13(5):188.
21. Marcickiewicz J, Blasiak J, Wysocka E, et al. A potential link between oral microbiota and female reproductive health disorders. *Microorganisms.* 2025;13(3):619.
22. Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information. *Database (Oxford).* 2010;2010:baq013.
23. Morrison AG, Sarkar S, Umar S, Lee STM, Thomas SM. The contribution of the human oral microbiome to oral disease: a review. *Microorganisms.* 2023;11(2):318.
24. Chakma A, Mishra SK, Roy S, et al. Homoeopathic management of polycystic ovarian syndrome: a case report. *AYU.* 2024.

25. Islam M, Chowdhury AK. Magic efficacy of Pulsatilla to treat polycystic ovarian syndrome. Indian J Integr Med. 2023.
26. Gunchala H. An experimental study to ascertain the effectiveness of Pulsatilla Nigricans in cases of polycystic ovary syndrome (PCOS). Int J Homoeopathic Sci. 2024;8(2):319-323. doi:10.33545/26164485.2024.v8.i2e.1151.
27. Raizada S. Homoeopathic management of polycystic ovarian syndrome--a case series. Indian J Res Homoeopathy. 2022;16(3).
28. Rath P, Gautam P, Goswami AD, Jana SN. Polycystic ovarian syndrome and homoeopathic management: a case series. Indian J Res Homoeopathy. 2024;18:200-211.
29. Masroor D, Rizwan A, Ahmad F, et al. Pharmacoeconomic evaluation of treatments for polycystic ovary syndrome. Resour Alloc. 2024.
30. Chikramane PS, Suresh AK, Bellare JR, Kane SG. Extreme homeopathic dilutions retain starting materials: a nanoparticulate perspective. Homeopathy. 2010;99(4):231-42.
31. Chikramane PS, Kalita D, Suresh AK, Kane SG, Bellare JR. Why extreme dilutions reach non-zero asymptotes: a nanoparticulate hypothesis based on froth flotation. Langmuir. 2012;28(45):15864-75.