**Microbial composition across body sites in PCOS: a systematic review and meta-analysis**

**ABSTRACT**

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disease in women of reproductive age, whose aetiology remains still unclear. Recent evidence is linking microbial composition in different body sites with PCOS, nevertheless the studies are barely comparable and results inconsistent. The aim of this systematic review and meta-analysis was to clarify the relationship of the microbiome of different parts of the human body with PCOS. For this purpose, a systematic search in main databases such as PubMed, Web of Science, Scopus, Cochrane Library, PROSPERO, medRxiv and bioRxiv was carried out up to April 2022. Although the evidence associates some changes in the microbiome with PCOS, the heterogeneity of the studies, the small sample size, the lack of adequate controls, and the possible effect of confounders, make difficult to establish a clear relationship. Based on our meta-analysis of the gut microbiome data from 1868 women (737 women with PCOS and 631 controls) demonstrate decreased gut microbiome diversity compared to controls, which may contribute to PCOS development. Future studies are needed to determine the mechanisms by which microbes may alter/modulate the symptomatology and progression of this metabolic disorder.

**INTRODUCTION**

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous endocrine disorder that affects up to 20% of reproductive-aged women worldwide (Goodarzi *et al.*, 2011), being one of the most prevalent gynaecological disorders. Several diagnosis criteria have been suggested but clinical and/or biochemical hyperandrogenism, oligoanovulation and polycystic ovaries (the presence of ≥12 follicles with maximum diameter of 2–9 mm or any ovarian volume >10 mL) are the key criteria, and the presence of two of the above conditions are considered sufficient for the diagnosis (The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Additionally, other features such as hirsutism, acne, alopecia, menstrual dysfunction, metabolic syndrome, cardiovascular disease, hypertension, endometrial cancer, and endometrial receptivity alteration could be related to PCOS (Escobar-Morreale, 2018). Despite greats efforts to PCOS diagnosis and its high prevalence, the aetiology of PCOS remains widely unknown (Dokras *et al.*, 2017).

Recent evidence is linking microbial composition in different body sites with various diseases, including PCOS (Molina *et al.*, 2020; Hill and Round, 2021).Several microbiota (collection of microorganisms) and microbiome (genomes of the microorganisms) studies are highlighting the role of microorganisms in human physiology and pathology by both the direct or indirect interactions with host cells, modulating our metabolism, immune system and therefore our state of health (Power *et al.*, 2017; Altmäe *et al.*, 2019; Laniewski *et al.*, 2020).

Microbial communities may be influenced by several factors such as diet, physical activity, cultural habits, host genetics and hormones, among others (Molina *et al.*, 2020). Hormones are crucial in microbial function and composition (Wilson *et al.*, 2007; Baker *et al.*, 2017). Likewise, microbes could influence the microenvironment by the production of several metabolites like bile acids, ceramides, short-chain fatty acids, branched-chain amino acids and trimethylamine N-oxide (Chen and Pang, 2021). Therefore, given the endocrine aetiology of PCOS, it seems reasonable to investigate the potential role of microorganisms in this pathology. The aim of this systematic review and meta-analysis was to gather the knowledge and raise the power for analysing the relationship between the microbiome composition in different body sites with PCOS.

**METHODS**

*Bibliography search strategy*

The search strategy was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page *et al.*, 2021). The review protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO, CRD42020180191). We performed a systematic search of the literature available in PubMed, Web of Science and Scopus up to 19 April 2022. The terms were also indexed in Cochrane Library, PROSPERO, medRxiv and bioRxiv to ensure the up-to-date data. The search approach was performed pairing/combining the terms “polycystic ovary syndrome”, “polycystic ovary disease”, “PCOS”, “PCOD” with terms “microbiome”, “microbiota”, “microorganism”, “microbes”, “infection”, “bacteria”, “virus”, “flora”, “microflora”.

*Selection criteria*

The study population consisted of women with and without diagnosed PCOS. The inclusion criteria were as follows: all case-control studies that compared the microbiome between women with and without PCOS. The exclusion criteria were: 1) conference abstracts, letters to editors, study protocols or review articles and 2) studies written in any language other than English and Spanish. The outcomes of interest were microbial diversity, abundance/richness, and changes in specific taxa of any human tissue/body site.

The resulting studies from the systematic search were screened by title and abstract by two independent researchers (ASL and NMM) and possible discrepancies were discussed and solved by a third independent researcher (SA). For every eligible study, data extraction was performed including the following information: 1) authors’ name and bibliographic reference; 2) cohort’s characteristics (number of study subjects, condition, age, ethnicity, exclusion criteria, and PCOS criteria); 4) sample collection (body niche, type of sample); 5) detection of microorganisms (DNA extraction, detection method); 6) main findings; and 7) raw data availability.

*Quality assessment and risk of bias*

To evaluate the internal quality and possible bias in the study design of the selected works, two researchers (ASL and NMM) independently used the Joanna Briggs Institute Critical Appraisal Tool for Systematic Reviews (Moola *et al.*, 2015). Specifically, checklist for case-control studies was used, which consist of 10 items assessing the potential risk of bias for each study: 1) Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?; 2) Were cases and controls matched appropriately?; 3) Were the same criteria used for identification of cases and controls?; 4) Was exposure measured in a standard, valid and reliable way?; 5) Was exposure measured in the same way for cases and controls?; 6) Were confounding factors identified?; 7) Were strategies to deal with confounding factors stated?; 8) Were outcomes assessed in a standard, valid and reliable way for cases and controls?; 9) Was the exposure period of interest long enough to be meaningful?; 10) Was appropriate statistical analysis used? (Aromataris and Munn, 2020). Each researcher considered each item in detail and reported an overall evaluation. Possible inconsistencies were resolved through common agreement. A risk score was calculated by dividing the number of positively scored criteria by the total number of criteria. Low risk of bias was considered when the study achieved at least 75% of the items listed, otherwise the study was categorised as high risk of bias.

*Meta‐analysis of the microbiome studies*

A meta-analysis was conducted to assess the associations of microbial composition with PCOS. While microbiome studies of all body sites were included into the systematic search, meta-analysis was only performed on the gut microbiome studies, as studies on other body sites were scarce. Based on the available data of microbial diversity metrics, our meta-analysis focussed on Shannon diversity and Chao1 indexes from different gut microbiome studies. A random-effect model was performed using the Comprehensive Meta-Analysis software (version 3; Biostat Inc.,1385, NJ, USA). Statistical heterogeneity across studies was assessed using the I2 value, considering 25%, 50%, and 75% as low, moderate, and high heterogeneity, respectively (Higgins *et al.*, 2003). The effect size was calculated as standardised mean difference (SMD) based on Cohen’s d and 95% confidence intervals (CIs). Specifically, when SMD<0, the control group showed a higher alpha-diversity compared to PCOS group, and when SMD>0, a higher alpha-diversity was detected in the PCOS group.

It is important to highlight the following considerations in our meta-analysis: 1) only those studies that compared PCOS patients with body mass index (BMI)-matched controls or adjusted by BMI were included in the meta-analysis for discarding the effect of weight on microbiome data; 2) at least three studies were required to perform the meta-analysis as this is the minimal number recommended for a meta-analysis (Higgins *et al.*, 2003); 3) the Web-PlotDigitizer 4.4 software (Ankit Rohatgi [https://automeris.io/WebPlotDigitizer/]) was used to calculate the effect size for those studies that did not report alpha-diversity indexes in their manuscript (i.e., mean and standard deviation [SD]) and did not provide the data after request. This software allowed us to estimate the mean and SD from graphs reported in the article or, otherwise, the median, interquartile range (IQR) and maximum and minimum values, which were subsequently transformed into mean and SD by Wan method (Wan *et al.*, 2014). Web-PlotDigitizer is a web-based plot digitizing tool for extracting data from plots and has proven valid and reliable (Knowles *et al.*, 2016; Drevon *et al.*, 2017; Dhakal *et al.*, 2018; Bjørnelv *et al.*, 2022). The Wan method allows to estimate the mean and SD by incorporating the sample size, median, IQR and maximum and minimum values, demonstrating more accurate estimations when compared to other methods (Weir *et al.*, 2018).

**RESULTS AND DISCUSSION**

*Overall characteristics of selected studies*

A PRISMA flowchart of search strategy and selection of studies included in this work is shown in Figure 1. A total of 2839 studies were found across the databases and were examined by tittle and abstract. After exclusion, a full-text review was carried out for 52 studies. Finally, 33 studies met the inclusion criteria. Of these, 17 studies were selected for the meta-analysis (see Supplementary Table SI).

Regarding the risk of bias, over half of the studies identified in the systematic literature search (18/33, 54.4%) presented high risk of bias, and out of the 17 studies included into the meta-analysis 10 categorised as with high risk of bias (Supplementary Tables SII). This quality assessment clearly highlights that a big part of the microbiome studies conducted lack rigorous study design, especially in the aspect of properly matched cases and controls and consideration and controlling for confounders (Supplementary Table SII). It has been shown that body weight can alter gut microbial composition (Dominianni *et al.*, 2015), thus, BMI-matched controls would provide clearer information of the microbial changes in association to PCOS excluding the effects of obesity/overweight on microbiome. Confounders, other important points to consider in microbiome studies, is shown to result in spurious relations between the condition and the results if not properly taken into account (Lv *et al.*, 2021).

**Diagrama

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**Figure 1**. PRISMA flowchart of the systematic literature selection. Created with BioRender.

*Microbiome of the oral cavity*

Oral microbiome constitutes an important component of the microenvironment in human body and recently, oral cavity has been established as a potential source of microbes that could affect intestinal homeostasis and lead to inflammatory diseases (Atarashi *et al.*, 2017; Read *et al.*, 2021). Growing evidence links oral and salivary microbiome with PCOS. According to our systematic search, 4 studies have described the microbiome of the oral cavity in relation to PCOS (Akcali et al., 2014; Lindheim et al., 2016; Belkova et al., 2020; Wendland et al., 2020; Li et al., 2021) (Supplementary Table SI, Figure 2). The female sex hormones have been associated with the composition of oral microbiome linked to oral pathology, such as periodontal diseases (Kumar, 2013). The study of the most common bacterial pathogens that specifically cause gingivitis in women with and without PCOS revealed that there were not differences in microbial abundances directly linked to PCOS (Akcali *et al.*, 2014). However, the study of the oral microbiome composition in relation to PCOS by using metagenomic approaches reported that PCOS women had a decreased relative abundance of *Actinobacteria* (Lindheim *et al.*, 2016) and increased *Fusobacterium* (Li *et al.*, 2021). In terms of diversity metrics, there is no consensus so far about the differences between PCOS and controls women. Interestingly, the variation of the oral microbiome over time in patients with PCOS has been recently analysed and it revealed that PCOS and controls could be differentiated by their oral microbiome at different time-points (Li *et al.*, 2021). For a comprehensive understanding of the influence of oral microbiome in PCOS bigger-size studies are required, and exhaustive oral analysis including oral health factors should be included as potential confounders.

*Blood microbiome*

Actual evidence is showing that blood harbours its own microbiome and that variation in blood microbial composition could be linked to non-infectious-diseases (Amar *et al.*, 2013; Potgieter *et al.*, 2015; Lelouvier *et al.*, 2016; Païssé *et al.*, 2016). We found one study where the relationship between blood microbiome and PCOS was assessed (Wang et al., 2022). These preliminary results detected a decreased alpha diversity of blood microbiome in PCOS women and beta-diversity analysis showed dissimilarities between microbial communities of PCOS and controls women. The relative abundance of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* decreased significantly, while *Actinobacteria* increased significantly in PCOS women compared to controls (Wang *et al.*, 2022). Deeper understanding of blood microbiome related to PCOS aetiology should be addressed in bigger studies.

*Lower genital tract microbiome*

Altered lower genital tract microbiome in PCOS could be driven by the changes related to menstrual cycle and hormone levels (Song *et al.*, 2020). Based on our research, 4 studies have analysed the relationship between female reproductive tract microbiome and PCOS (Yeow *et al.*, 2016; Hong *et al.*, 2020, 2021; Tu *et al.*, 2020; Lu *et al.*, 2021) (Supplementary Table SI, Figure 2). Overall, these studies reported that PCOS women presented a vaginal microbiome dominated by *Mycoplasma* (Hong *et al.*, 2020; Tu *et al.*, 2020), *Prevotella* (Hong *et al.*, 2020; Tu *et al.*, 2020), *Gardnerella* (Tu *et al.*, 2020), *Actinomyces*, *Enterococcus* and *Atopobium* (Lu *et al.*, 2021). Regarding Lactobacilli species, women with PCOS showed less abundance when compared with controls (Hong *et al.*, 2020; Tu *et al.*, 2020). Also, a cross sectional study evaluated the presence of bacterial vaginitis and vulvovaginal candidiasis in a cohort of 89 women with PCOS revealed that approximately 15% of women presented microbial pathologies (Hong *et al.*, 2021).

*Gut microbiome*

The pivotal presence of insulin resistance and chronic inflammation in most of women with PCOS prompted Tremellen and Pearce in 2012 to propose a new paradigm for PCOS related to dysbiosis of the intestinal microbiome (Tremellen and Pearce, 2012). Since then, several studies have analysed the association between intestinal microbiome and PCOS (Supplementary Table SI, Figure 2). Nevertheless, a clear cause-effect relationship has not been established yet and the gut microbial diversity indexes in PCOS is still controversial. Although different studies have demonstrated a decreased alpha diversity and differences in beta diversity analyses, many other studies have not detected significant changes (Supplementary Table SI). Since the lack of consensus results could be influenced, in addition to study protocol, by the study size, it is worthy to mention that the biggest study up to now with 102 PCOS women and 201 age- and BMI-matched control women did not detect any significant differences in diversity indexes neither in microbial composition (Lüll *et al.*, 2020). Despite the discrepancies across studies, there seem to be some consensus on several microbial taxa being more prevalent among PCOS women: *Bacteroides* (Torres *et al.*, 2018; Qi *et al.*, 2019; Zeng *et al.*, 2019; Chu *et al.*, 2020; Haudum *et al.*, 2020), *Parabacteroides* (Zhang *et al.*, 2019; Chu *et al.*, 2020), *Prevotella* (Zhang *et al.*, 2019; Liang *et al.*, 2020), *Megamona* (Haudum *et al.*, 2020; Liang *et al.*, 2020), *Megasphaera* (Haudum *et al.*, 2020), *Escherichia* (Liu *et al.*, 2017; Chu *et al.*, 2020) and *Shigella*, while *Bifidobacterium* (Zhang *et al.*, 2019), *Lactobacillus* (Liu *et al.*, 2017) and *Faecalibacterium* (Zhang *et al.*, 2019; Chu *et al.*, 2020) genera seem to be less prevalent (Figure 2).

Among the clinical manifestation of PCOS, obesity raises as the most prevalent and some studies have shown that abdominal obesity is associated with clinical parameters of PCOS (Chen and Pang, 2021). In terms of microbial diversity in the gut, no differences between obese PCOS patients and lean PCOS women were found (Liu *et al.*, 2017; Insenser *et al.*, 2018; Zhou *et al.*, 2020). However, several studies are proving that gut microbiome in women with PCOS could be affected by obesity, increasing the abundance of *Bacteroides* spp., *Candidatus* (Insenser *et al.*, 2018)*, Lachnoclostridium*, *Fusobacterium*, *Coprococcus*\_2, and *Tyzzerella* (Zhou *et al.*, 2020). To determine the association between PCOS and microbiome composition, it is recommended to use BMI-matched controls to exclude the potential alteration driven by obesity on microbial composition.

Insulin resistance in terms of fasting glucose and insulin levels also conform an important alteration in PCOS patients. Microbial dysbiosis could be involved in insuline resistance via endotoxemia, some gut-brain peptides, hyperandrogenism and some abnormal metabolites (He and Li, 2020). Some trials performed in humans have analysed the influence of insulin resistance revealing that microbial families such as *Lachnospiraceae* and *Ruminococcaceae* had higher abundance in PCOS patients without insuline resistance. Also, Zeng and coworkers reported that women with insuline resistance and PCOS showed lowest number of observed bacterial taxa and a lower Shannon index (Zeng *et al.*, 2019).

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**Figure 2**. Microbial composition in women with PCOS in oral cavity, blood, gut and female reproductive tract. Created with BioRender.

*Meta-analysis of the gut microbial diversity in PCOS*

Alpha diversity is considered a good indicator of the gut microbiome health and, particularly, PCOS has been associated with a decrease in alpha diversity (Thackray, 2019). So far, many studies have reported a decreased alpha diversity of the gut microbiome in women with PCOS, however, some studies have not detected such differences (Guo *et al.*, 2022). The studies are with different study design and often conducted in a small sample size, therefore a meta-analysis could increase the power in clarifying whether there are differences in microbial composition in PCOS.

Figure 3 illustrates the meta-analysis of the PCOS *vs.* BMI-matched controls’ effect on both alpha diversity metrics, i.e., Shannon diversity and Chao1 indexes. Specifically, 14 studies (624 cases and 573 controls) were eligible for the first meta-analysis focussed on Shannon diversity index, while 9 (394 cases and 228 controls) were included in the second meta-analysis based on Chao1 index. A significant pooled SMD was found for Shannon diversity index, indicating a significantly higher richness in the control group compared to PCOS group (SMD= -0.204; 95% CI -0.360- -0.048; p= 0.010; I2= 5.508). Contrastingly, no significant differences were found for Chao1 index, although a tendency to a higher Chao1 index favouring controls was observed (SMD= -0.153; 95% CI -0.475- 0.170; p= 0.353; I2= 0.000).



**Figure 3.** Forest plots of alpha-diversity metrics including Shannon diversity (A) and Chao1 (B) indexes in PCOS patients and healthy controls. Pooled effect size was estimated using a random-effects model. Each point represents standardised mean difference (SMD) and 95% confidence interval (CI). +PCOS patients with normal-weight *versus* healthy controls with normal-weight; \*PCOS patients with overweight/obesity *versus* controls with overweight/obesity. Torres *et al*. 2018, Lüll *et al*. 2020, Li *et al*. 2021 and Zhu *et al*. 2021 included participants of different BMI categories, however, there were no significant differences between groups, or adjusted by BMI if applicable.

*Inflammatory state, microbiome and PCOS*

Supporting the hypothesis presented in 2012 by Tremellen and Pearce, disorders in the gut microbiota of women with PCOS could increase mucosal permeability and lead to increased surrounding lipopolysaccharides (LPS) levels in the blood resulting in systemic inflammation mediated by C reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor α (TNFα), among others. The increased inflammation can interfere with insulin signalling promoting ovarian hyperandrogenism (Tremellen and Pearce, 2012). Therefore, PCOS has been acknowldeged as part of the systemic chronic low-grade inflammation syndromes (Duleba and Dokras, 2012). The study conducted by Zeng et al. demonstrated that the levels of CRP, IL-6 and TNFα were higher in PCOS patients compared with healthy controls, and levels of these biomarkers were negatively associated with the abundance of *Prevotella* (lower abundant in PCOS women) (Zeng *et al.*, 2019). Another study correlated the levels of zonulin (i.e., modulator of intercellular tight junctions) with microbial alpha diversity, pointing out that changes in the integrity of the intestinal epithelium is directly connected with the microbiome and with the inflammatory state produced by PCOS (Lindheim *et al.*, 2017). Even the evidence prompts to confirm the effect/cause PCOS-microbiome relationship, there are not enough studies in the field.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

It is clear that microbes have important role in human health and disease and there is growing body of evidence that microbes play a role in the aetiolgoy of PCOS. Different body sites have been analysed for the microorganismal composition, such as oral cavity, whole blood, vagina and the gut, and although novel, the studies across different sites in women with PCOS are scarce, except for the gut. The studies possess different limitations, such as a low number of participants and differences in age, ethnicity, and BMI as well as other non-controlled variables that need to be taken into account for the study of the microbiome (Molina *et al.*, 2020), making difficult to reach any solid conclusions whether the microbiome is different in women with PCOS *vs.* controls.

With our systematic review, we gathered microbiome data of 1868 women, 737 women with PCOS and 631 control women in order to raise the power for clarifying whether there are changes in the microbial diversity and composition in PCOS. As the majority of studies in the field have analysed the gut microbiome, we were able to meta-analyse microbiome diversity data from 624 cases and 573 controls for Shannon diversity index, and 394 cases and 228 controls for Chao1 index, making it the biggest analysis conducted. Our study results support the concept of the decreased gut microbial diversity in PCOS, which may contribute to PCOS development. Although the cause and/or causality need to be established in the future studies. The results here presented support the hypothesis previously proposed by Larsen et al. which argues that greater diversity leads to greater stability of the microbial system that is associated with redundancy (Larsen and Claassen, 2018). The protective/damaging role of the gut microbiome in metabolic functions, and therefore in PCOS, was evident when transplants of the gut microbiome from obese mice into normal mice induced an increase in body fat and resistance to insulin (Turnbaugh *et al.*, 2008). Also, as letrozole model causes hyperandrogenism in the mice, it is likely that the changes in microbiome are linked to steroid hormone functions as has also been shown in other animal models, linking androgens and gut microbiome in the occurrence of diabetes (Markle *et al.*, 2013).

Our study also demonstrates the shortcomings in the study design in the microbiome analyses, highlighting the need for well-planned and conducted studies with bigger sample size, proper negative and positive controls, control for important confounders and proper case-control matching. Future studies are needed to determine the mechanisms by which microbes may alter/modulate the symptomatology and progression of this common metabolic disorder.

**SUPPLEMENTARY MATERIAL**

The supplementary files can be downloaded at this link: <https://osf.io/yrbqh/>

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