Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Obstetrics and Gynecology Research

Journal homepage: www.ijogr.org

Original Research Article

Types of vaginal microbiomes in PCOS affected females

Dalya Thamer Ahmed [1,*]

[1] *Al-Iraqia University / College of Medicine, Baghdad, Iraq*

ARTICLE INFO

Article history:
Received 18-06-2021
Accepted 26-06-2021
Available online 26-11-2021

Keywords:
Poly cystic ovarin syndrom (PCOS)
Vaginal microbiota
Rotterdam Criteria and Lactobacilli
Speceies

ABSTRACT

Objective: To evaluate and analyze the microbiomes component of the vagina in females with polycystic ovarian syndrome PCOS, and compare it with that of healthy females.
Study Design: A case- control study included 120 participants, 60 had been diagnosed as having PCOS according to the Rotterdam Criteria for diagnosis of PCOS and the other 60 are healthy females visiting the outpatient private clinics in Hay Aljameaa/ Al-Harthya in Baghdad from October 2020 till march 2021 for different medical problems, statistical analysis was done by using the SPSS computer application for statistical analysis.
Results: Both study groups had L. crispatus in their vagina, while for L. jensonii 93.33% of control group have this microbiota while only 66.66% of PCOS group have it, L. gasseri presents in the vagina of 80% of controls and only 38.33% of PCOS. S. aureus in 41.66% of PCOS group and only 3.33% of control group, S. epidermidis presents in 25% of PCOS females while it not presents in control group. Str. Pyogenes presents in 36.67% of PCOS group and absent in control group ($p< 0.0001$), Str. Agalactiae presents in 26.67% of PCOS group and 1.67% of control group. Bacteroides presents in 30% of PCOS cases and only in 1.67% of controls ($p< 0.0001$). For other types of vaginal microbiota e.g. Gardnerella vaginalis we found that it presents in high percentage of PCOS group 66.67% and absent in females of control group, Prevotella spp presents in 55% of PCOS group and only 3.33% of control group, Mobiluncus spp and Fusobacterium spp were absent in both study groups. For candida species, C. albicans presents in 30% and 6.67% of vagina of PCOS and control group respectively.
Conclusion: There is large diversity in the vaginal microbiota with disruption to normal flora in PCOS affected patients so We need further studies to evaluate the relationship between the microbiota and different PCOS symptoms.

1. Introduction

Polycystic ovary syndrome, is a heterogeneous and complex issue that affects women's reproductive and metabolic health.[1,2] It is considered to be the most prevalent female endocrine abnormalities.[3] Although there is a geographical variation in the incidence of PCO but the incidence rate is thought to be (3-10%).[3] PCOS has an effect on many facets of a woman's physical wellbeing, including long-term consequences that extend much further reproductive age.[1]

The cause of PCOS is unknown; however, various factors have been reported as contributing to the development of a hormonal and metabolic dysfunction that can contribute to the onset of this syndrome.[4] A few studies suggest that alterations in the microbiome may be contributed in the genesis of PCOS.[1] The most widely accepted hypothesis is that PCOS is a genetically determined syndrome, but with clinical and biochemical variability which is determined by the combination of genetic and environmental influences.[5] Menstrual irregularity,

* *Corresponding author*.
E-mail address: d.alimohamad11@gmail.com (D. T. Ahmed).

https://doi.org/10.18231/j.ijogr.2021.093

hyperandrogenism, and polycystic ovary morphology are some of the parameters used to diagnose PCOS (PCOM). (6) According to NIH 2012/International PCOS Guidelines 2018, the involvement of at least two of the following three criteria must be present in order to diagnose PCOS: oligo- and/or anovulation, hyperandrogenism (clinical or biological), and polycystic ovaries.[6]

PCOS has a significant influence on subsequent women's health because it triggers several metabolic disorders such as insulin resistance (IR), diabetes, and obesity.[7]

When studying the pathogenesis of PCOS, it's critical to look at alterations in metabolic states in a comprehensive approach.[8] Although latest study demonstrates that Gut Microbiomes disturbances are connected to the onset and progression of metabolic diseases,[9] but the correlation between different vaginal microbiome and the etiology and pathogenesis of PCOS is poorly understood.[10]

The human microbiome includes all parasitic microorganisms found on the skin, throughout the digestive, respiratory, and urogenital tracts.[11] The vaginal microbiota have a positive interaction with their host and have a significant influence on health and disease.[12] Considering their significance, little is understood about how these groups vary in function and structure between individuals, and, more specifically, how their constituent representatives communicate with one another and the host to form a complex ecosystem that responds to environmental disruptions. Major attempts are also being made to help recognize the true nature of these groups and their role in health and disease prevention.[12] PCOS women with varying clinical manifestations have different vaginal bacterial strains.[13]

2. Materials and Methods

A case-control study in which the microorganisms settling in the vaginal area were compared for two groups of women, the first group suffering from PCOS and the second group being the control group.

Women of both groups were recruited at the outpatient private clinics in Hay Aljameaa/ Al-Harthya in Baghdad between October 2020 and March 2021. Data that were collected included demographic data, detailed information's about signs and symptoms of PCOS, full medical, surgical, family, social and drug history.

2.1. Study population and exposure variable

All women in both groups of reproductive age, divided into 2 groups first group includes PCOS patient and the second group includes women without PCOS presents to outpatient clinics for other complains like vaginal discharge, the Rotterdam Criteria were used to diagnose PCOS,

Diagnosis of PCOS, requiring two of three features: anovulation or oligo-ovulation (irregular menstrual periods (oligomenorrhoea or amenorrhea)), clinical and/or biochemical hyperandrogenism, and polycystic ovaries by ultrasound.[14]

2.2. Ethical consideration

Informed consent was obtained from each participant. The significant and purpose of the study was explained to women. Confidentiality of any obtained information was ensured.

2.3. Exclusive criteria

For both groups, all patients with endocrinal abnormalities like thyroid disorder, diabetes, hyperprolactinemia, Cushing's syndrome and cancer were excluded. Pregnant, lactating and menstruating women were also excluded. All patients any antibiotics oral or vaginal for the last 7 days before the test, no hormonal treatment and no sexual relationship within 48 hours.

For the control group, healthy women whose age were similar age as the PCOS patients were selected. They visit outpatient clinic for fertility problems or contraceptive purposes. All their physical examination indexes were normal.

2.4. Statistical design

Data was collected, coded, tabulated and analyzed, using the SPSS computer application for statistical analysis. Descriptive statistics was used to calculate percentages and frequencies.

2.5. Sample collection

Collection of swabs was done under complete aseptic technique, high vaginal swabs HVS were gathered at the day of patient visit for outpatient clinic.

After opening of swab package, the swab shaft was hold in the middle without touching the tip of the swab, then it inserted about 5-7 cm in the vagina carefully and rotate for 20-30 seconds, after the swab absorbed the moisture from vaginal wall, it withholds without touching the skin of the perineum. Vaginal swabs were immediately placed in a clean tube containing 0.5 ml sterile saline. Samples were placed immediately in a refrigerator or ice bucket at 4- 8°C and then at -20°C in less than 4 hours. All the instruments used in these steps were sterilized.

2.6. Laboratory methods

The Vitek 2 Compact (30 card capacity) system can identify organism by utilizing a fluorogenic methodology also can test susceptibility by using a turbidimetric method depending on a 64 barcoded well card with information on card type, lot number and unique card identification number and expiration date. ID-GN (gram negative bacillus

identification), ID-GP (gram positive cocci identification), AST-GN (gram negative susceptibility), and AST-GP (gram positive susceptibility) are some of the test kits available.

Within 10 hours, the Vitek 2 ID-GN card can identify 154 Enterobacteriaceae species and a limited set of glucose non-fermenting gram negative organisms. Within 8 hours or fewer, the Vitek 2 ID-GP card can identify 124 staphylococci, streptococci, enterococci, and a limited set of gram-positive organisms.[15]

3. Results

The difference between the two proportions and a 95% confidence interval (CI) for this difference; the CI is calculated, Chi-squared test and P value: when this P value is less than 0.05, the conclusion is that the two proportions indeed differ significantly.

Total number of cases enrolled into the study is 120 case, 60 control and 60 PCOS case.

In Table 1 we compare the sociodemographic data, the mean age of PCOS patient is (27.3±951) while mean age of control group is (29.1±0.786), (3.33%) were smokers in PCOS group and (1%) in controls, the PCOS patient tends to be obese (mean of BMI is 27.1±4.22) while the mean of BMI for control group is (22.5±2.12)

In Table 2 sign and symptoms of PCOS and control group, we found that there is significant difference in mean of the frequency of menstrual cycle between both study groups (5.5±1.5) and (11.3±1.6) respectively (p-value < 0.0001, 58 cases of total 60 PCOS cases were have oligomenorrhea while 1 out of 60 control group have oligomenorrhea. All PCOS cases had Ultrasound feature of polycystic ovaries while control group have no case have this feature; for biochemical and clinical feature of hyperandrogenism, p-value was significant between both study group.

The Table 3 shows the comparison between microbial component of the vagina in both study groups, for the Lactobacilli species, we see that all females in both study groups had L. crispatus in their vagina, while for L. jensonii 93.33% of control group have this microbiota while only 66.66% of PCOS group have it, L. gasseri presents in the vagina of 80% of controls and 38.33% of PCOS.

Atopobium vaginae was found by Rodriguez in 1999 as a frequent vaginal commensal,[16] this microbiota was found in about 75% of PCOS group and 30% of control group.

For Staphylococcus species, we found that S. aureus in 41.66% of PCOS group and only 3.33% of control group, S. epidermidis presents in 25% of PCOS females while it not presents in control group.

Peptostreptococcus is a common bacterium seen in women's lower reproductive tracts,[17] it was seen in 13.33% of PCOS group and only 1.66% of control group (p =0.0156).

For Streptococcus species, er found that Str. Pyogenes presents in 36.67% of PCOS group and absent in control group (p< 0.0001), Str. Agalactiae presents in 26.67% of PCOS group and 1.67% of control group.

Bacteroides presents in 30% of PCOS cases and only in 1.67% of controls (p< 0.0001)

For other types of vaginal microbiota e.g. Gardnerella vaginalis we found that it presents in high percentage of PCOS group 66.67% and absent in females of control group, Prevotella spp presents in 55% of PCOS group and only 3.33% of control group, Mobiluncus spp and Fusobacterium spp were absent in both study groups.

Escherichia coli presents in vagina of 23.33% of PCOS group and 11.67% of that of control group, Mobiluncus spp, Fusobacterium spp and Klebsiella pneumoniae were all absent from vagina of control group but presents in vagina of PCOS group 8.33%, 3.33% and 1.67% respectively.

Diphtheroids presents in vagina of 10% of PCOS, 3.33% of control group (p=0.1447), while Chlamydia trachomatis and Neisseria gonorrhoeae were absent from vagina of both study groups.

Trichomonas vaginalis presents in 5% and 3.33% of vagina of PCOS and control group respectively.

For candida species, C. albicans presents in 30% and 6.67% of vagina of PCOS and control group respectively, while both C. glabrata and C. tropicalis were absent in vagina of control group and only 5% of PCOS group (p== 0.0807).

4. Discussion

In our study we found that both groups of the study had abundant Lactobacillus species in their vagina as most of similar studies found that in the majority of women, those species are the most common vaginal bacteria.[18,19]

L.crispatus was found in all participants of both groups, but its concentration may be altered as many other microbiotas was detected in the HVS of the first group, those microbiotas was not detected in control group, L. jensonii and L. gasseri was detected in more frequent in control group than PCOS group, this result is similar to result of other study done by Xiang Hong et al. in 2020[19] and Yaoyao Tu and et al. 2020.[20]

The study of vaginal inhabitants of PCOS patients and healthy controls shows that PCOS patients' microbiomes are more diversified, so in agreement with previous recent studies done in 2020 and 2021, this study found that unlike control group, the PCOS group had many other types of microbiotas were presents in their vagina e.g. Atopobium vaginae,[13] Streptococcus species,[13] Gardnerella vaginalis,[13,20] Prevotella species[13,19,20] and Mycoplasma.[13,19,20]

Unlike other study we find frequent detection of Candida albicans in PCOS patient swaps this may be explained by glucose intolerance or insulin resistance precipitate

Table 1: Demographic characteristic of study groups

Characteristic	PCOS (n= 60) No. (%) or Mean ± SD	Control (n = 60) No. (%) or Mean ± SD	Mean difference or odds ratio (95% CI) *	p-value
Age	27.3±951	29.1±0.786	1.4846 to 2.1154	< 0.0001
Smoking	2(3.33%)	1(1.66%)	-5.9157% to 9.8136%	0.5603
BMI	27.1±4.22	22.5±2.12	-5.8073 to -3.3927	< 0.0001

Table 2: PCOS signs and symptoms of study groups

	PCOS (n= 60) No. (%) or Mean ± SD	Control (n = 60) No. (%) or Mean ± SD	Mean difference or odds ratio (95% CI) *	p-value
Frequency of menstrual cycle/ 1 year	5.5±1.5	11.3±1.6	5.2393 to 6.3607	< 0.0001
Oligomenorrhea hyperandrogenism	58(96.66%)	1(1.66%)	84.2225% to 97.7779%	< 0.0001
Testosterone level (nmol/L)	2.8±0.6	1.4±0.5	-1.5997 to -1.2003	< 0.0001
Acne	23(38.33%)	4(6.6%)	17.1054% to 44.9489%	< 0.0001
Hirsutism	35(58.33%)	5(8.33%)	34.0734% to 62.5260%	< 0.0001
Alopecia	2(3.33%)	0	-3.1528% to 11.3590%	0.1558
Ultrasound feature of polycystic ovaries	60(100%)	0	91.4904% to 100.0000%	< 0.0001

Table 3: Prevalence of organisms diagnosed from HVS

	Organisms	PCOS (n= 60) No. (%)	Control (n = 60) No. (%)	Mean difference or odds ratio (95% CI)	p-value
	Lactobacilli				
1	L.crispatus	60(100%)	60(100%)	-6.0172% to 6.0172%	0
2	L. jensonii	40(66.66%)	56(93.33%)	12.5913% to 39.9129%	= 0.0003
3	L. gasseri	23(38.33%)	48(80%)	24.3839% to 55.5702%	< 0.0001
4	Atopobium vaginae	45(75%)	18(30%)	27.5045% to 58.6792%	< 0.0001
	Staphylococci				
5	S. aureus	28(41.66%)	2(3.33%)	24.2210% to 51.1642%	< 0.0001
6	S.epidermidis	15(25%)	0	13.9873% to 37.2321%	< 0.0001
7	Peptostreptococcus spp.	8(13.33%)	1(1.66%)	2.0361% to 22.5872%	= 0.0156
	Streptococci				
8	Str. pyogenes	22(36.67%)	0	24.0913% to 49.3203%	< 0.0001
9	Str. agalactiae	16(26.67%)	1(1.67%)	13.0580% to 37.4185%	= 0.0001
10	Bacteroides	18(30%)	1(1.67%)	15.9305% to 40.9140%	< 0.0001
11	Gardnerella vaginalis	40(66.67%)	0	52.6982% to 77.2736%	< 0.0001
12	Prevotella spp.	33(55%)	2(3.33%)	36.8068% to 63.8183%	< 0.0001
13	Mobiluncus spp	0	0	-6.0172% to 6.0172%	
14	Fusobacterium spp	0	0	-6.0172% to 6.0172%	
15	Escherichia coli	14(23.33%)	7(11.67%)	-2.1098% to 25.1242%	= 0.0942
16	Mycoplasma	5(8.33%)	0	0.6825% to 18.0647%	= 0.0230
17	Ureaplasma	2(3.33%)	0	-3.1528% to 11.3590%	= 0.1558
18	Coryneforms (Diphtheroids)	6(10%)	2(3.33%)	-2.9702% to 17.1023%	= 0.1447
19	Klebsiella pneumoniae	1(1.67%)	0	-4.5021% to 8.8604%	= 0.3168
20	Trichomonas vaginalis	3(5%)	2(3.33%)	-7.0050% to 10.6988%	= 0.6484
	Candida spp				
21	C. albicans	18(30%)	4(6.67%)	9.6264% to 36.4765%	= 0.0010
22	C. glabrata	3(5%)	0	-1.8555% to 13.7005%	= 0.0807
23	C. tropicalis	3(5%)	0	-1.8555% to 13.7005%	= 0.0807
24	Chlamydia trachomatis	0	0	-6.0172% to 6.0172%	
25	Neisseria gonorrhoeae	0	0	-6.0172% to 6.0172%	

by PCOS pathology that provide a good environment for candida for growth and multiplication. Streptococcus pyogenes, Str. Agalactiae and Bacteroides were also detected significantly in swaps of PCOS patients that other studies failed to detect.

In this study we did not detect Chlamydia trachomatis or Neisseria gonorrhoeae in both groups of the study, this result not agreed with Yaoyao and et al study who found abundant Chlamydia trachomatis in the PCOS group, this difference in the result may be explained by the cultural, social and ethnic diversity between both communities of the studies in spite of the closer number of sample size.

In this study we detected many other microbiotas e.g., Coryneforms (Diphtheroids), Klebsiella, Ureaplasma, Escherichia coli and Trichomonas vaginalis although the difference is not statically significant between both study group but these results reflect the huge variation of the vaginal inhabitants and disruption of vaginal flora in PCOS group.

5. Conclusion

There is large diversity in the vaginal microbiota with disruption to normal flora in PCOS affected patients so we need further studies to evaluate the relationship between the microbiota and different PCOS symptoms.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

References

1. Giampaolino PI, Foreste V, Filippo CD, Gallo A, Mercorio A, Serafino P, et al. Microbiome and PCOS: State-of-Art and Future Aspects. *Int J Mol Sci*. 2021;22(4):204.
2. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome. *Endocr Rev*. 2015;36(5):487–525. doi:10.1210/er.2015-1018.
3. Wolf WM, Wattick RA, Kinkade ON, Olfert MD, Int J Environ Res Public Health. Geographical Prevalence of Polycystic Ovary Syndrome as Determined by Region and Race/Ethnicity. *Int J Environ Res Public Health*. 2018;15(11):2589. doi:10.3390/ijerph15112589.
4. Giampaolino P, Corte LD, Rosa ND, Mercorio A, Bruzzese D, Bifulco G. Ovarian volume and PCOS: A controversial issue. *Gynecol Endocrinol*. 2017;34:229–32.
5. Leo VD, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol*. 2016;14(1):38. doi:10.1186/s12958-016-0173-x.
6. Draft Summary and Recommendations of International Evidenced-Based Guideline for the Assessment and Management of Polycystic Ovary Syndrome; 2018. Available from: https://www.monash.edu/__data/assets/pdf_file/0004/1412644/PCOS_Evidence-Based-Guidelines_20181009.pdf.
7. Teede HJ, Misso ML, Costello MF. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum Reprod*. 2019;34:388.
8. Zhao X, Jiang Y, Xi H, Chen L, Feng X. Exploration of the Relationship Between Gut Microbiota and Polycystic Ovary Syndrome (PCOS): a Review. *Geburtshilfe Frauenheilkd*. 2020;80(2):161–71. doi:10.1055/a-1081-2036.
9. He Q, Li X, Liu C. Dysbiosis of the fecal microbiota in the TNBS-induced Crohn′s disease mouse model. *Appl Microbiol Biotechnol*. 2016;100:4485–94.
10. Hong X, Qin P, Huang K, Ding X, Ma J, Xuan Y, et al. Association between polycystic ovary syndrome and the vaginal microbiome: A case-control study. *Clin Endocrinol (Oxf)*. 2020;93(1):52–60.
11. Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: networks, competition, and stability. *Science*. 2015;350:663–6.
12. Ma B, Forney LJ, Rave J. The vaginal microbiome: rethinking health and diseases. *Annu Rev Microbiol*. 2012;66:371–89.
13. Hong X, Qin P, Yin J, Shi Y, Xuan Y, Chen Z, et al. Clinical Manifestations of Polycystic Ovary Syndrome and Associations With the Vaginal Microbiome: A Cross-Sectional Based Exploratory Study. *Biomed Pharmacother*. 2021;133:110958.
14. Wang R, Mol BWJ. The Rotterdam criteria for polycystic ovary syndrome: evidence-based criteria? *Hum Reprod*. 2017;32(2):261–4.
15. Gundersen Health System, Standard Operating Procedure. Vitek 2 Compact – Identification and Susceptibility Testing; 2019. Available from: https://www.gundersenhealth.org/app/files/public/4608fc82-436a-481a-b59f-da2bc1fdb31c/Lab-Policies-Vitek-2-Compact---Identification-and-Susceptibility-Testing-Lab-1512.pdf.
16. Jovita MR, Collins MD, Sjödén B, Falsen E. Characterization of a novel Atopobium isolate from the human vagina: description of Atopobium vaginae sp. nov. *Int J Syst Bacteriol*. 1999;49(4):1573–6.
17. Hoffman B. Williams gynecology. 2nd ed. New York: McGraw-Hill Medical; 2012. p. 65.
18. Ma B, Forney LJ, Rave J. The vaginal microbiome: rethinking health and diseases. *Annu Rev Microbiol*. 2012;66:371–89.
19. Hong X, Qin P, Huang K, Ding X, Ma J, Xuan Y, et al. Association between polycystic ovary syndrome and the vaginal microbiome: A case-control study. *Clin Endocrinol (Oxf)*. 2020;93(1):52–60.
20. Tu Y, Zheng G, Ding G, Wu Y, Xi J, Ge Y, et al. Comparative Analysis of Lower Genital Tract Microbiome Between PCOS and Healthy Women. *Front Physiol*. 2020;11:1108. doi:10.3389/fphys.2020.01108.

Author biography

Dalya Thamer Ahmed, Assistant Professor https://orcid.org/0000-0001-7566-0861

Cite this article: Ahmed DT. Types of vaginal microbiomes in PCOS affected females. *Indian J Obstet Gynecol Res* 2021;8(4):443-447.