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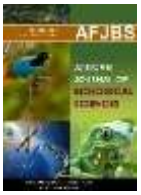

Identification and evaluation of AMR strains from Polycystic Ovarian Syndrome (PCOS) through metagenomic approach.

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Abstract : Polycystic Ovarian Syndrome (PCOS) is a hormonal disorder affecting numerous women of reproductive age. Emerging evidence suggests a potential association between PCOS and the development of antimicrobial resistance (AMR) in the gut microbiota. This study aims to employ a metagenomic approach to identify and evaluate AMR strains in women with PCOS. The identification of AMR strains in PCOS patients would provide insights into the association between PCOS and AMR development , leading to potential interventions and treatments.

Keywords: Polycystic Ovarian Syndrome , metagenomics, gut microbiota, AMR strains

Introduction: Polycystic Ovarian Syndrome (PCOS) is a complex hormonal disorder affecting approximately 5-10% of women of reproductive age. It is characterized by various symptoms, including irregular menstrual cycles, increased androgen levels and the formation of cysts on the ovaries. Recent research suggests a potential link between PCOS and changes in the gut microbiota, including the presence of antimicrobial resistance (AMR) strains. Understanding the role of AMR strains in PCOS can contribute to the development of targeted interventions and AMR management strategies.Identifying and evaluating AMR strains in PCOS patients is crucial for understanding the potential link between PCOS and AMR development. Studies have shown alterations in the gut microbiota of women with PCOS ,including changes in bacterial composition and increased levels of certain bacteria associated with AMR.

The metagenomic approach can be employed to analyze the bacterial communities present in the gut from PCOS patients. Metagenomic sequencing allows for the identification and evaluation of AMR genes and strains by analyzing the genetic material extracted from the samples. This approach provides a comprehensive view of the microbial composition and the presence of AMR strains in PCOS patients. The results of a metagenomics analysis of blood samples from PCOS patients may reveal the presence and prevalence of AMR strains in the gut microbiota. These findings can help establish a potential association between PCOS and AMR development. Additionally, identifying specific AMR genes and strains in PCOS patients can aid in the development of targeted interventions and treatment strategies. It is important o note that more research is needed in this field to fully understand the role of AMR strains in PCOS. Larger cohort studies with control groups and long-term follow- ups are necessary to elucidate the casual relationship between PCOS and AMR development in the gut microbiota. Nonetheless,the identification and evaluation of AMR strains in PCOS patients through a metagenomic approach provide valuable insights into this potential association and pave the way for future research and interventions to mitigate AMR in PCOS patients.While looking at the gut microbial diversity in the community level, the phyla *Firmicutes, Bacteriodetes, Actinobacteria* and *Verrucomicrobia* are the most dominant in the healthy gut. Phyla like *Proteobacteria, Escherichia* and *Shigella* were positively associated with PCOS (Rizk MG et al). According to the metagenomic species studies (MGS),Thirty nine MGS showed a negative correlation with reproductive hormones and glucose metabolism. *Enterobacteriaceae* is positively correlated with testosterone, anti-mullerian hormone and luteinizing hormone in the PCOS patients. When compared with that of the control group, the MGS related to the control group were negatively correlated with the serum levels of testosterone(Chu W et al). Excess androgen production and relative lower estradiol production are two important factors associated with the PCOS and the follicle development in the ovaries.A previous study also shows that *Lactobacillus* plays an important role in maintaining good health. Patients with decreased estradiol levels exhibited low levels of *Lactobacillus* in their gut. When the patients were administered with *Lactobacillus,* the patients showed significant increase in the estradiol levels(Guo Y et al) .At the phyla level, studies showed that abundance of *candidatus Saccharibacteria* was higher in obese patients than in non- obese patients. This phylum plays an instrumental role in the degradation of different organic compounds as well as compounds like glucose , butyrate , oleic acid and amino acids(Insenser M et al). Gut microbiota affects bile acid metabolism and lead to insulin resistance. Certain studies revealed that the *Bacteriodes* in rhe intestinal composition of microorganisms of PCOS patients increased significantly, which may be caused by the decrease in the levels of IL- 22 , insulin resistance and PCOS by affecting thee bile acid synthesis mechanism(Liu R et al) .This study aims to identify and evaluate the antimicrobial resistance strains from PCOS patients . By understanding it , we can gain valuable insights and provide effective treatment plans for the patients .

2. METHODOLOGY:

2.1 Study design :

This study design will employ a case control design recruiting 20 PCOS patients and age matched 20 healthy individuals as controls.

2.2 Sample collection :

Blood samples collected from participants following standardized procedures to preserve microbial DNA integrity. Ethical committee approval obtained .

2.3 Metagenomic Sequencing:

High – throughput metagenomic sequencing performed to analyse microbial diversity and identify AMR genes within the guy microbiota . It is a powerful technique used to analyse the genetic material from complex microbial communities directly extracted from environmental or clinical samples , such as soil , water , feces or biological tissues. This technique provides insights into the composition, diversity and functional potential of microbial communities without the need for culturing individual organisms.

2.3.1 DNA extraction :

Total DNA is extracted from the collected samples using specialized kits and techniques optimized for extracting DNA from diverse microbial populations. The extracted DNA should be of high quality and free from contaminants that could interfere with downstream sequencing.

2.3.2 Library Preparation:

Extracted DNA undergoes fragmentation into smaller , manageable fragments using physical or enzymatic methods.
DNA fragments are then located with adapters that contain sequences necessary for the subsequent steps of sequencing, such as binding to the sequencing platform and indexing for multiplexing (sequencing multiple samples in a single run)

2.3.3 Sequencing :

The prepared DNA libraries are subjected to sequencing using high throughput sequencing platforms such as Illumina , Ion Torrent , or PacBio . During sequencing, DNA fragments are amplified and sequenced in parallel, generating millions of short reads representing the genetic information within the sample .

2.3.4 Data processing and Quality Control:

Raw sequencing data undergo initial quality control steps to remove low- quality reads , adaptors and sequencing artifarcts . Quality filtered reads are then processed to remove host DNA and other contaminating sequences , leaving behind microbial DNA reads .

2.4 Bioinformatic Analysis :

DNA fragments are then located with adapters that contain sequences necessary for the subsequent steps of sequencing, such as binding to the sequencing platform and indexing for multiplexing (sequencing

multiple samples in a single run) . Bioinformatic tools and databases utilized to annotate sequencing data, identify AMR genes , and assess their prevalence and distribution.

2.4.1 Taxonomic profiling:

Reads are compared against databases (NCBI ‘s BLAST) to identify and classify microbial taxa present in the sample.

2.5 Metagenomic data interpretation:

Results from taxonomic and functional analyses provide insights into the microbial composition, diversity, relative abundance of taxa , and potential metabolic functions within the studied ecosystem. Comparative analyses between different samples (PCOS patients vs healthy controls) can reveal microbial signatures, dysbiosis patterns, and potential associations with disease states or environmental factors.

2.6 Data visualization and reporting:

Results are often visualized through Krona charts to represent microbial community structures and functional profiles.

3. RESULTS :

The 16S metagenomic results of 20 patients with PCOS were compared with 20 age matched heathy individual controls . The lysed blood was used to identify the total microbial DNA which was then extracted and sequenced. The reads were taxonomically identified using NCBI’s BLAST software . Individual metagenomic data were visualized with a Krona chart diagram. From the Krona chart , we identified that *Prevotella* (19 %) , *Bacteroidaceae* (7%) , *Phacaeicola dorei* (2%) , *Alistepes* (0.8 %) to be significantly present in the PCOS patients as compared to the heathy controls. The healthy controls Krona chart , significantly represented *Oscillospiraceae* (30%) , *Bifidobacterium* (6%) , *Proteobacteria* (2%) , *Firmicutes* (5%) which were not found in the Krona chart of PCOS patients.

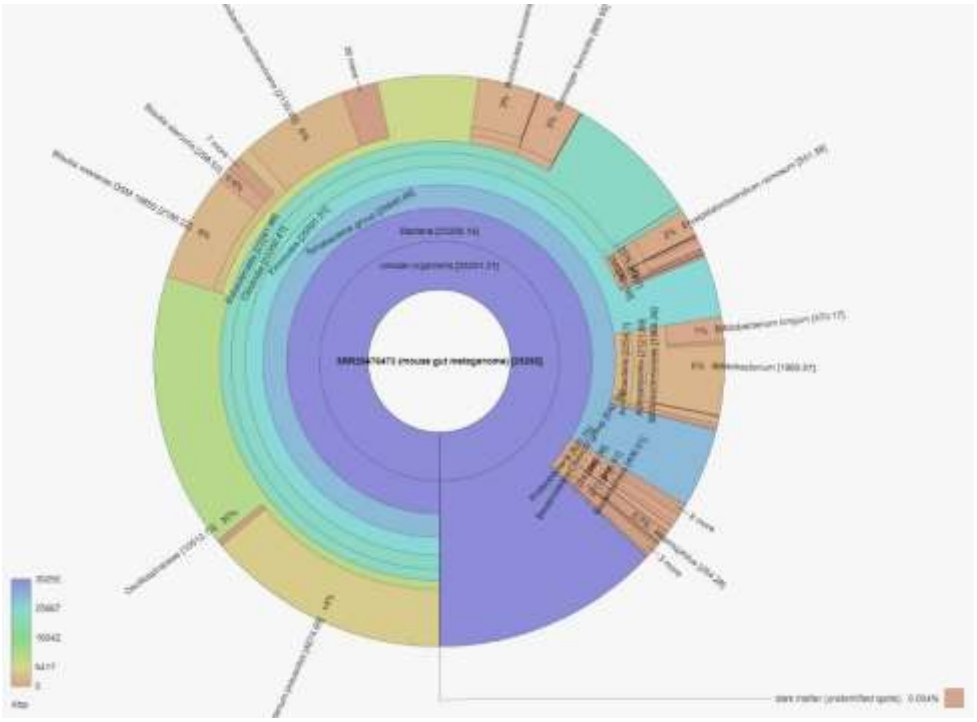

Fig.1. Krona chart depiction of healthy gut microbiome

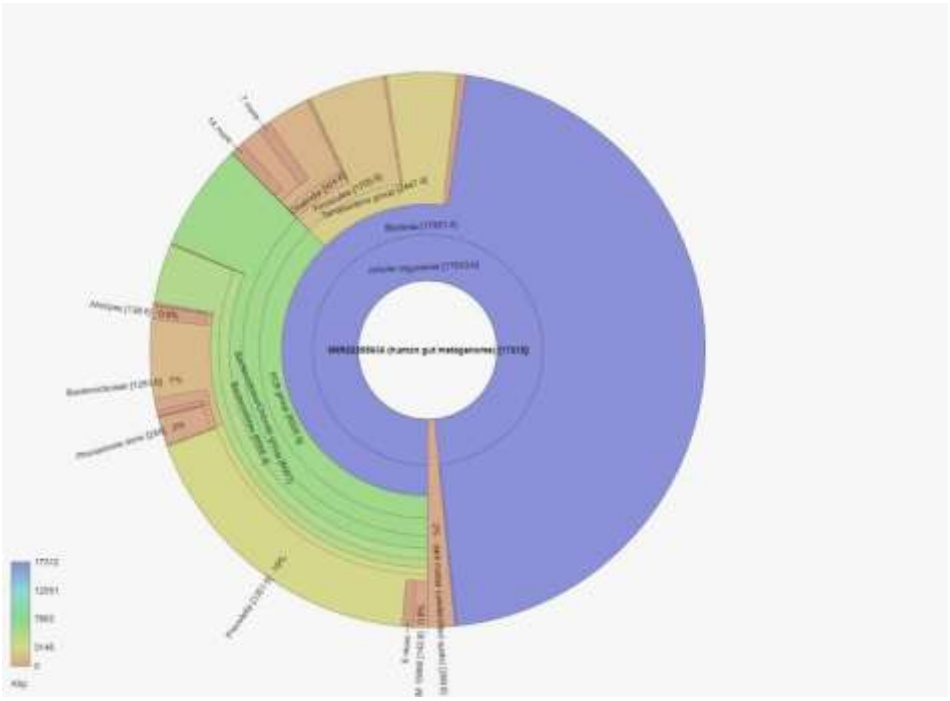

Fig 2 . Krona chart depiction of PCOS patients gut microbiome

4. DISCUSSION :

PCOS is increasingly recognised as a condition influenced by microbial dysbiosis, including alterations in gut microbiota composition and function. The metagenomic approach allows for a comprehensive analysis of the microbial communities , including bacteria , fungi , viruses and their genetic potential providing a deeper understanding of microbial contributions to PCOS pathogenesis. Similar to (Haudam C et al), the PCOS group showed less microbial diversity as compared to the control group. The comparison made here will help elucidate whether PCOS associated microbial communities exhibit distinct AMR profiles and if these profiles contribute to the disease phenotype. Understanding AMR strains in PCOS patients has direct clinical infor antibiotic selection, treatment efficacy, and management strategies. Unlike (Bai X et al), our study showed no correlation between *Prevotella* and BMI. According to (Gan J et al) it is found that probiotics were increased after treatment in both groups. Clostridium was lower than the control group before treatment, but decreased after treatment. In (Yang Z et al),Seven microbial genera—Blautia, Coprobacillus, Actinomyces, Pseudomonas, Enterococcus, Erysipelatoclostridium, and Gordonibacter—were substantially enriched in PCOS among the genera with > 0.05% of abundance. The PCOS patients gut microbiome was found to be lack of *Proteobacteria* , which exhibit diverse metabolic functions ; *Oscillospiraceae* , which help in the fermentation of dietary fibers and complex carbohydrates producing short chain fatty acids such as acetate , propionate and butyrate , which are important for gut health , energy metabolism and immune regulation; *Firmicutes* , which help in maintaining gut barrier function, reducing inflammation and promoting gut health. The PCOS patients gut microbiome was found to be rich in *Prevotella* , which is usually said to be linked to certain immune responses and inflammatory conditions like inflammatory bowel disease (IBD) ; *Bacteriodaceae* , have been associated with IBD, obesity , metabolic syndrome and diabetes in abundance; *Alistepes* , has been found to be associated with colorectal cancer, obesity and metabolic syndrome in abundance.

5. CONCLUSION:

Our study identified novel AMR mechanisms and less studied resistance genes within the PCOS associated microbiota. These findings pave the way for further investigations into the genetic context , transmission dynamics, and functional implications of these resistance determinants. In conclusion our metagenomic analysis provides comprehensive insights into the AMR landscape of PCOS. It is crucial for optimizing therapeutic interventions and mitigating the challenges posed by antimicrobial resistance in PCOS management. Strategies aimed at promoting a balanced and diverse gut microbiota , such as dietary modifications , prebiotics and probiotics may help in PCOS management by altering the guy microbiota. This research also suggests avenues for future research and intervention related to AMR in PCOS

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