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RESEARCH ARTICLE

Core Gut Microbiome in Patients with Staghorn Calculi in Malaysia

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ABSTRACT - Globally, staghorn calculi stone prevalence is 3–5% annually, with a lifetime prevalence of up to 25%. Recurrence rates are 10% within the first year, 50% within 5–10 years, and 75% over 20 years, despite improvements in treatment. This study aimed to profile the gut microbiota in 77 participants, including 37 staghorn calculi patients and 40 healthy individuals, to identify biomarkers linked to staghorn calculi formation. At the phylum level, *Firmicutes A* and *Proteobacteria* were more abundant in the staghorn group, comprising 52.86% and 13.65%, respectively. At the species level, staghorn patients had lower levels of *Bifidobacterium adolescentis* and higher *Escherichia* and *Megamonas funifomis* abundances compared to controls. Core microbiome analysis identified *Blautia A* as the most abundant species in healthy individuals, whereas *Megamonas funiformis* was exclusive to staghorn patients, suggesting its potential as a disease biomarker. LEfSe analysis confirmed *Megamonas funiformis* as significantly enriched in staghorn patients (LDA score: -4.46). These findings highlight critical gut microbiome differences that may contribute to staghorn calculi development.

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1. INTRODUCTION

Staghorn calculi, a severe and complex form of kidney stone disease, account for 10–20% of all nephrolithiasis cases worldwide, posing a significant health burden [1]. Globally, kidney stone prevalence is 3–5% annually, with a lifetime prevalence of up to 25% [2]. In Malaysia, the prevalence of kidney stones has risen dramatically, from fewer than 40 per 100,000 residents to 442.7 per 100,000 in recent years [3]. This alarming trend highlights the need for a deeper understanding of the disease's underlying mechanisms to develop innovative management strategies.

Staghorn calculi are large, branched kidney stones that can obstruct the renal pelvis, major calyces and minor calyces, potentially causing kidney failure [4]. While staghorn stones typically occur in one kidney, they can affect both kidneys in some cases. These stones account for 10-15% of urinary calculi in developing countries and are more prevalence in women [5]. Although surgical interventions like percutaneous nephrolithotomy (PCNL) are considered the gold standard for treatment, recurrence rates remain high—10% annually and up to 75% over 20 years [6]. Numerous bacterial species, including *Oxalobacter formigenes*, have been implicated in stone formation or preventive measure [7]. However, no particular bacterial species yet been found to be exclusively in charge of the formation of staghorn calculi stones, particularly in the Malaysian population, hence, highlighting the needs for research to characterize the specific microbes involved in advanced stone formation. In Malaysia, while extensive research has been conducted on various aspects of staghorn calculi by the usage of urinary microbiota of healthy individuals to determine the risk factors of renal stones, there is a notable gap in investigating the gut microbiome's role in staghorn calculi formation using stool samples [8].

Emerging research has highlighted the pivotal role of the gut microbiome in metabolic processes, particularly in calcium oxalate metabolism, which is closely linked to kidney stone formation [9]. This study aims to address the gap in profiling the diversity of gut microbiota and its association with staghorn calculi. Using amplicon sequencing technology, specifically Illumina sequencing, the gut microbiome of staghorn calculi patients and healthy individuals will be profiled to identify the core microbiome and potential microbial biomarkers. The research will focus on identifying microbial species that could serve as biomarkers for disease diagnosis and progression and by optimizing library preparation for the detection of microbial communities in staghorn calculi patients' samples using Illumina technology.

By identifying specific microbial species and understanding the core microbiome composition, this study aims to provide valuable insights into the pathogenesis of staghorn calculi, facilitating early diagnosis, improved risk assessment, and the development of personalized treatment strategies [10]. This novel approach holds the potential to enhance the management of staghorn calculi, reducing recurrence rates and enabling targeted preventative measures.

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2. METHODS AND MATERIAL

2.1 Sample Collection and Preparation

Stool samples were collected from participants at Sultan Ahmad Shah Medical Centre, comprising 37 staghorn calculi patients diagnosed with urinary tract stone disease. The study was approved by the Kulliyyah of Medicine Research Committee, International Islamic University Malaysia (004/2019), and informed consent was obtained from all participants. Samples were stored in sterile tubes at 4 °C to preserve integrity and prevent microbial growth. Before analysis, the frozen samples were thawed at 4°C, resuspended in a 1:2 ratio with autoclaved distilled water, vortexed for 5 minutes, and centrifuged at 13,500 rpm for 2 minutes to obtain a pellet for further processing.

2.2 DNA Extraction, Library Preparation, and Sequencing

Microbial DNA was extracted from stool pellets using the Qiagen PowerLyzer™ PowerSoil® DNA Isolation Kit following the manufacturer's protocol [11]. The hypervariable V3 region of the 16S rRNA gene was selected for its taxonomic resolution. Primers were designed using Primer-BLAST, integrating consensus sequences from MEGALIGN 6.1, to target the V3 region across 16S rRNA gene variants. DNA quality and quantity were assessed using a Qubit 4 Fluorometer and agarose gel electrophoresis, ensuring sample integrity for sequencing. Libraries were prepared using the Nextera XT Index Kit, where DNA was fragmented, and adapter sequences were ligated for amplification. The prepared libraries were quality-checked and sequenced on the Illumina MiSeq platform, which uses flow-cell technology to produce high-throughput sequencing data with precise base calling, enabling comprehensive characterization of microbial communities.

2.3 Raw Microbial Genome Data Analyses

The raw sequencing data in FASTQ format were processed using the QIIME2 pipeline to identify amplicon sequence variants (ASVs). Initial quality assessment was conducted to evaluate sequencing accuracy and to inform decisions on trimming and filtering of low-quality reads. The DADA2 algorithm was used to denoise the data, remove chimeric sequences, and generate ASVs [12]. The resulting ASVs and feature tables were summarized to assess sequencing depth and microbial diversity. Taxonomic classification was performed by comparing the ASVs to the SILVA reference database, which facilitated the identification of microbial taxa present in each sample. The taxonomic profiles were visualized, providing insights into the composition and diversity of the microbial communities in the samples [13]-[15].

2.4 Statistical Analysis

Alpha diversity indices, including the Shannon and Observed indexes, were calculated to measure within-sample microbial diversity. Beta diversity was assessed using Jaccard Index and Bray-Curtis dissimilarity matrices to evaluate inter-sample differences [16-17]. Microbiome Analyst and LEfSe were employed for biomarker discovery, identifying taxa with significant differences in abundance between groups ($p < 0.05$) [18]. These analyses provided insights into microbial community composition and potential biomarkers associated with staghorn calculi disease.

3. RESULTS AND DISCUSSION

In this study, the gut microbiota of individuals diagnosed with staghorn calculi is examined, data profiling based on variations in bacterial composition between healthy individuals and staghorn calculi patients was conducted. The V3 region in the complete 16S rRNA region was sequenced using high-throughput amplicon sequencing by Illumina platform to determine the composition of bacteria in the gut microbiota of 37 staghorn calculi patients. Raw sequences were demultiplexed, clipped, and quality filtered during pre-processing stage [19]. After post-processing, a total of 781 ASV with an average of 68,937 read counts per sample were obtained.

3.1 Community Profiling of Gut Microbiome

At the phylum level, the staghorn calculi patient group exhibited notable dysbiosis, with a shift in microbial composition. *Firmicutes_A* dominated the microbiome at 52.86%, along with a marked enrichment of *Proteobacteria* (13.65%), suggests an inflammatory gut environment. Depletion of *Firmicutes_D* (8.61%) and an increase in minor phyla like *Firmicutes_C* (7.62%) and *Bacteroidota* (5.60%) further indicates a disruption in the microbial balance. These changes are associated with a decrease in microbial diversity, a hallmark of dysbiosis observed in staghorn calculi patients (Table 1).

Escherichia and *Megamonas funiformis* showed marked increases in the staghorn calculi group. The rise in *Escherichia* indicates a potential shift towards an inflammatory gut environment, as certain strains are linked to inflammation and infection, which could contribute to the formation of struvite stones [20]. Similarly, the increase in *Megamonas funiformis*, involved in protein breakdown, points to altered protein metabolism in these patients [21]. These changes in microbial species highlight a potential imbalance in the gut microbiome, which may play a role in the pathophysiology of staghorn calculi (Table 2).

Table 1. Major differences in phylum level between the microbial diversity of healthy individuals and staghorn calculi patients

Taxonomic abundance of species	Staghorn calculi individuals (%)
p__Actinobacteriota	9.98
p__Proteobacteria	13.65
p__Firmicutes_C	7.62
p__Firmicutes_D	8.61
p__Bacteroidota	5.60
p__Firmicutes_A	52.86
p__Verrucomicrobiota	1.59
p__Desulfobacterota_I	0.07

Table 2. Major differences in top 10 species between the microbial diversity of healthy individuals and staghorn calculi patients across 30 species

Taxonomic abundance of species	Staghorn calculi patients (ASV)
s__Bifidobacterium_adolescentis	9063
g(U)_Escherichia	45595
s__Megamonas_funiformis	24154
g(U)_Blautia_A	81612
g(U)_Faecalibacterium	30811
g(U)_Collinsella	11031
g(U)_Bifidobacterium	13349
s__Bifidobacterium_longum	3807
f(U)_Lachnospiraceae	51252
s__Holdemanella_biformis	2431

3.2 Core Microbiome

Core microbiome analysis is essential for identifying consistent microbial features across populations, providing insights into baseline microbial composition and its alterations in disease development [22]. A color gradient from dark blue (low prevalence) to dark red (high prevalence) represents relative abundance. In staghorn calculi patients (Figure 1), *Blautia A* remains dominant. A key observation in patients is the presence of *Megamonas funiformis*, which is either absent or negligible in healthy individuals, suggesting it may be a potential biomarker for staghorn calculi. Additionally, at the medium threshold, *g(U)_Ruminococcus_E* and *f(U)_Peptostreptococcaceae* emerge as important indicators in the staghorn calculi group.

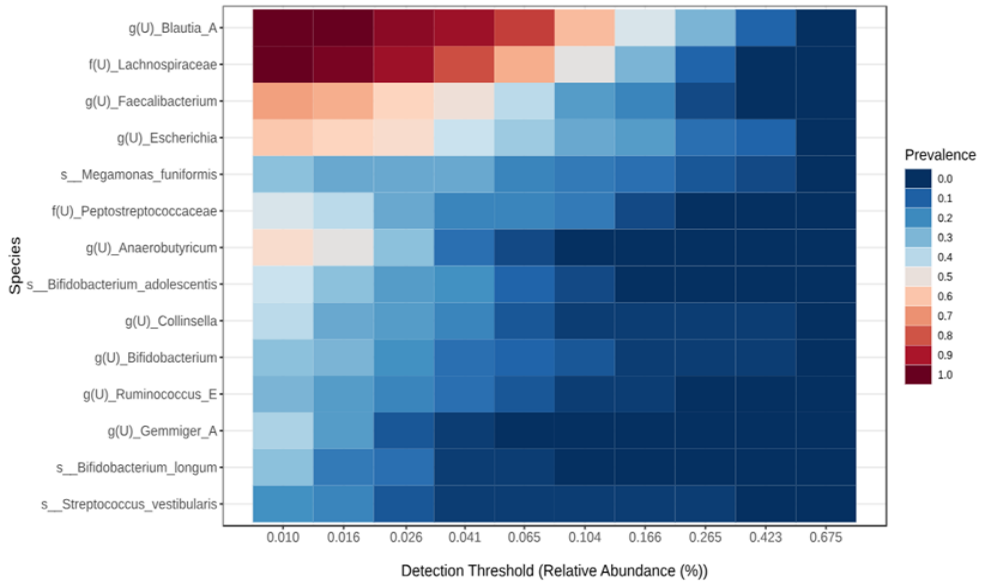

Figure 1. Core microbiome heatmap of staghorn calculi patients at 0.01% relative abundance and 20% sample prevalence

4. CONCLUSION

In conclusion, *Megamonas funiformis* formed core microbiome for staghorn calculi. However, further research is needed to elucidate their roles in disease etiology and their potential as diagnostic or therapeutic targets, validating the findings of this research using larger and more diverse populations to establish the reliability of *Megamonas funiformis* as biomarkers for staghorn calculi. Mechanistic investigations are required to understand the roles of these gut microbes in stone formation. Longitudinal studies tracking microbial changes over time and the impact of interventions on gut microbiota are recommended. Additionally, exploring microbiota-targeted therapies may provide innovative approaches to managing urolithiasis. Finally, integrating microbiome profiling into diagnostic frameworks could enhance early detection and facilitate personalized treatment strategies for staghorn calculi.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

C. H. Yin (Formal analysis; Writing - original draft)

H. F. Ahmad (Conceptualisation; Methodology; Resources; Investigation; Data curation; Validation; Writing - review & editing)

M. N. Kamarulzaman (Resources; Software; Visualisation; Supervision)

S. L. Liew (Writing - review & editing)

M. S. M. Coba (Formal analysis; Writing - original draft)

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