Abstract of the Thesis

Oxalic acid (Ethanedioic acid: C₂H₂O₄) is a metabolite produced in the systemic fluid, and its elevated level results in hyperoxaluria. The emergence of calculi in the urinary system is considered to be a symptomatic phase, may lead to the chronic disease to the human. Similar to urea and uric acid, accumulation of oxalate is also considered as a uremic toxin for an excretory system. It can alter the renal cell functions that sometimes leads to the differential expression of abnormal proteins in nephritic cells. Thus, in hyperoxaluric condition gut lumen acts as a primary excretory system to remove excessive oxalate. To maintain a reductive level of oxalate content, gut-inhabitants (gut microbiome) have an exquisite role. Such functional gut microbes called as Oxalate Metabolising Bacterial Species (OMBS) possess oxalate degrading enzymes which complement and helps oxalate metabolism in humans. OMBS through its enhanced excretion of degradative enzymes plays an active role in oxalate homeostasis in the gut and systemic fluids. The structural and functional composition of the gut microbiome and oxalate excretion level in the symptomatic phase of hyperoxaluria condition such association has yet been elusive. Nowadays, using new DNA sequencing technologies alterations in microbiota had been linked to various diseases. Where which we can use this methodology to detect gut microbiome diversity and can link it with the development of kidney stone disease.

In the present study, we assessed recurrent stone episode subjects (KSD) under ‘case-control study’ and found calcium oxalate (CaOx) as a principle component of surgically removed stones, low urine volume and high oxalate content in 24-hr urine (a day sample) in KSD. These findings can be considered as characteristics of hyperoxaluria condition in tested subjects. In Indian scenario, recurrent kidney stone disease is prevalent mainly due to hyperoxaluria. Growing evidence supports the fact that either microbial cells or their metabolic products have implications in the development of kidney stones. Because microbes may be involved in the formation of CaOx crystals in the urinary system, we analyzed surgically removed stones for the presence of microbes in the stone nidus using 16S rRNA gene clone library approach.
We were able to show the presence of genus mostly *Bacillus, Enterobacter, and Leucobacter*, etc. in the nidus of kidney stone, and may make them potential contributors to the development of oxalate kidney stones.

To study the differences between the compositions of gut microbiota among healthy control subjects (HLT) and recurrent kidney stone subjects (KSD), we used high-throughput DNA sequencing and traditional clone library approach. Comparative analysis of the Eubacterial diversity of KSD subjects revealed the abundance of Firmicutes, Proteobacteria and TM7 phyla, and depletion of Bacteroidetes and Cyanobacteria. Genus such as *Collinsella* increases and genus like *Bacteroides, Deinococcus*, and *Sutterella* are found to be decreased in KSD. KSD subjects on PCoA plots were found to be discrete compared to HLT subjects due to its unique microbiota. The inter-individual microbiome differences among the KSD subjects was observed with actual stone episodes and number of surgically removed kidney stones. Quantitative surveillance using qPCR assays was able to capture specific dysbiosis occurring in hyperoxaluric condition to support the DNA sequencing findings and showed quantitative perturbation in a specific range of OMBS from the human gut.

The well-known OMBS, *Oxalobacter formigenes*, varied from $3.2 \times 10^{05}$ to $8.9 \times 10^{06}$ and *frc*-gene copies (a molecular marker for OMBS diversity) in HLT subjects ranged from $7.8 \times 10^{05}$ to $7.7 \times 10^{07}$ counts per gram of stool which were significantly lower than its copies in KSD. The ratio of *frc*-gene copy number to 16S rRNA gene copy number observed much higher for KSD (range: 0.03 to 20.1 %) than in HLT subjects (range: 0.00018 to 0.01 %). This confirmed the fact that in diseased condition active OMBS are highly enriched. The ratio of *O. formigenes* in HLT found maximum 88.13 %, indicates that it is an important contributor for oxalate homeostasis in the gut of HLT. However, the significantly low ratio in KSD, 0.22 %, indicated its inhibition and associated enrichment of other OMBS in KSD subjects. Thus, we speculate that the OMBS diversity and their associated functional gene content (here *frc*-gene) as a constitutive component of the microbiome and may impart the substantial role in metabolic disorders.
Moreover, the colonization of *O. formigenes* along with *Lactobacillus plantarum* (*O-L colonization*) was found to be inversely associated with the hyperoxaluria condition. Additionally, it was recorded that *O-L colonization* were able to retain healthy microbiota in some of the KSD subjects. Targeted-functional metagenome using sequencing of *frc*-gene revealed that gut microbiota of hyperoxaluric subjects was found augmented with OMBS other than *O. formigenes* in the human gut. Another *but-, buk*-gene analysis (marker gene for short chain fatty acid producers) indicated butyrate-producing bacterial species is to be decreased in the disease condition.

Along with the Eubacteria, other microbiota components such as Archaea, Fungi, and Microeukaryotes were detected in KSD and HLT group subjects though the metagenome sequencing. We catalog these microbiome components to the species level, and interestingly some new taxa were found in the KSD subjects only. This suggests that increase in the probability of newer species augmentation as an effect of hyperoxaluria condition in the human gut.

In ecological niches especially terrestrial ecosystem, the oxalate-carbonate pathway is a part of the natural carbon cycle. Oxalate-carbonate pathway is known to be driven by the Oxalate Metabolising Bacterial Species (OMBS) and the plants only. To explore the OMBS diversity, we attempted its isolation from various ecological niches. We isolated taxonomically different OMBS and evaluated its oxalate metabolism activity. This successful attempt supports the fact that oxalate-carbonate pathway existed in their ecological niche, and the presence of OMBS reflects its active role. Considering the importance of OMBS in handling the oxalate in the gut, we aimed at isolation and characterization of some of the indigenous OMBS. To this end, we have successfully isolated the oxalate tolerating Lactic Acid Bacteria (*Lactobacillus plantarum* E2C2 and E2C5) from Indian healthy gut. The whole genome sequencing of these two isolates using Illumina Miseq platform was carried out and characterized them for the presence of promising genes for the betterment of human health. Phenotypic expression of gene products like cholesterol-lowering effect (*bsh*-gene activity) were tested in-vitro condition, these isolates which may be useful in defining ‘Generally Recognized as Safe’ (GRAS) probiotic candidate.
The present study concluded hyperoxaluria condition may acts as selection pressure in the gut and alters the microbial community, its ecological networks, and there is a selective enrichment of acid tolerant OMBS in the human gut. The study also reports the abundance of indigenous bacteria which can metabolize oxalate and can help in host oxalate homeostasis. These results suggest an important avenue be further explored for causality and possible interventions to prevent or modify the course of hyperoxaluria and related disorders. We anticipate the need for subsequent studies describing differences in gut microbial communities of kidney stone patients from different populations and identification of relevant population specific biomarkers. Further, upon validation of specific gut microbiota based biomarkers using qPCR could help in designing simple and convenient tools for disease diagnosis in future.