Chapter 6: Conclusion and Future Perspectives

6.1 Conclusion

Gut inhabitants and their metabolic processes are collectively responsible for the gut ecology maintenance in the human body. With the help of recent advent of sequencing technologies, we could able to deduce the ecological components and their metabolic potentials in particular health status. Strategies were useful to find the role and metabolic potentials of ecological components majorly for Eubacteria in the human gut when the gross comparatives in two different health status. The hyperoxaluria condition and the gut microbial community structural changes was the major aim of this study and findings presented in this thesis provide salient features of gut microbiota of apparently healthy subjects and structural and functional differences in gut microbial communities of hyperoxaluric subjects from India.

The data presented in this thesis, we came with the understanding for the gut microbiota and their components are found to disturbed in the hyperoxaluria condition. The ‘case-control study’ was based on the symptomatic recurrent kidney stone male patients. The surgically removed kidney stones were found to be pure with CaOx content. After 24-hr urine analysis and kidney stones from respective patient subjects (KSD), we can conclude that CaOx crystals in recurrent stone formers had characteristics of hyperoxaluria condition and had low urine volume and high oxalate content in 24-hr urine samples. Our observations in the form of bacterial imprint in CaOx stones suggests the involvement of bacteria in the stone nucleation and growth in the urinary system.

Eubacterial dysbiosis in gut inhabitants of healthy and hyperoxaluric subjects was observed. Higher abundance of Firmicutes and Proteobacteria and depletion in Bacteroidetes were the major findings. Substantial effects of hyperoxaluria conditions were recorded regarding the change in patterns of ecological interactions occurring in Eubacteria and solicited on certain genera which dwell positively or negatively with the urine oxalate concentration. Metagenomic imputation of Eubacteria depict deficiencies of various essential functions such as carbohydrate
metabolism, amino acid metabolism, butyrate production and increased oxalate metabolizing genes in hyperoxaluric subjects. Quantitative surveillance using qPCR assays, we were able to capture specific dysbiosis occurring in hyperoxaluric condition and showed quantitative perturbation in a specific range of OMBS from the healthy gut.

Moreover, the colonization of *Oxalobacter formigenes* a well-known OMBS was found inversely associated with the hyperoxaluria condition and sustained the healthy gut bacteria if found colonized. When looking at the indigenous oxalate metabolizers’ and oxalate utilization phenomenon has been found to be constitutive microbiome components. The targeted metagenome approach using *frc*-gene amplicon sequencing revealed that gut microbiota of hyperoxaluric subjects was found augmented with other OMBS along with *Oxalobacter formigenes* in the human gut. Butyrate-producing bacterial species are found to be decreased in the disease condition along with the Eubacteria other microbiota components Archaea, Fungi, and Microeukaryotes. These Trans-domain components were detected and identified it to the species level taxonomy. About 47 % identified species has been newly augmented, and about 22 % species were deprived in the disease condition. Thus, Trans-domain diversity analyses revealed probability of newer species augmentation as an effect of hyperoxaluria condition in the human gut.

Oxalate Metabolising Bacterial Species (OMBS) were isolated from various ecological niches which support the fact that Oxalate-Carbonate pathway existed in their ecological niche, and the presence of OMBS itself reflects this activity. Further, cultivation of facultative oxalate tolerating *Lactobacillus plantarum* E2C2 and E2C5 done from Indian healthy gut and its whole genome data detects most promising health beneficial genes which may be useful in defining ‘Generally Recognized as Safe’ (GRAS) probiotic candidature.

Our observations presented in this thesis rationalize the fact that hyperoxaluria acts as selection pressure in the gut and alters the Trans-domain community. There is dislodge in functional diversity in particular selective enrichment of acid tolerant OMBS in hyperoxaluria condition. The abundance and surveillance of indigenous
bacteria which can metabolize oxalate and help in host oxalate homeostasis can be the prognostic biomarkers for metabolic disease in the human gut. The validation of specific gut microbiota based biomarkers using qPCR could help in designing simple and convenient tools for disease diagnosis in future. And OMBS from healthy gut would be the promising candidates in remedial prospects.
6.2 Future Perspectives

6.2.1 Stool Bacterial Dysbiosis value Index as a Diagnostic Tool for Kidney Stone Disease

Urine has to be supersaturated to form stone with CaOx crystals inside the urinary system. The relative risk for stone formation was strongly correlated with urine calcium concentration. The pH of urine is another critical determinant of KSD recurrence. It is worthwhile to evaluate patients for underlying causes of stone formation. Urine analysis as prognostic purpose can be easily perceived but as a preventive therapy would more logical in further research. We speculate that through urine analysis along with stool bacteria detection from kidney stone sufferer will provide the exact idea about the recurrent rate in his each episodes. Thus, preventive therapy can be rendered which would decrease the recurrence to prevent more complicated surgical procedures and complicated medical issues with the chronic kidney disease.

**Hypothesis:** We hypothesized that the risk of oxalate nephrolithiasis in patients could be predicted by the numerical value: a ‘SbCO Index’ which is defined by analyzing these three parameters.

1. OMBS in stool examination,
2. Crystals in urine examination, and
3. Oxalic acid in urine examination.

**Explanation:** Bacterial colonization and ecology maintained by well-characterized OMBS, *Oxalobacter formigenes* and their presence may affects the different bacterial population inside the gut as a ‘colonization effect’ (Suryavanshi et al., 2016). Oxalobacter formigenes is known to have the major oxalate reducer inside the human gut. Its colonization is found to be stable and prevalence found to be less than 50 % in the American (Barnett et al., 2016) and more than 65 % to the 100 % in Indian population (Kumar et al., 2002; Suryavanshi et al., 2016). *Oxalobacter* colonization has been limited by the oxalate concentration in the urine and in systemic fluids (Kwak et al., 2003). *Oxalobacter formigenes* along with other OMBS
are found enriched when gut ecology was supplemented with the oxalate (Miller et al., 2016). The OMBS like Lactobacillus plantarum has found the equal relation to kidney stone disease, i.e. its colonization is negatively correlated to the hyperoxaluria condition. The dysbiosis or change in the colonization status of both these bacteria inside the gut speculated to be the firmly associated to the hyperoxaluria condition and may directly correlate to the stone recurrent rates.

In other hand, urinary saturation is calculated using the computer based program EQUIL2 (Werness et al., 1985), and the BONN-Risk-index (Laube et al., 2014) is used for disease diagnosis. The measurement of plasma oxalate and urinary glycolate helps to distinguish between primary and secondary hyperoxaluria. If secondary hyperoxaluria is suspected, the stool is examined for Oxalobacter formigenes, an intestinal oxalate degrading bacterium, as lack or absence may lead to increased intestinal oxalate absorption (Hoppe et al., 2003).

In light of this hypothesis (Figure 6.1), we can conclude that SbCO value index can be a major indicator for presence and recurrence of kidney stone disease in patients and correctional strategy by bacterial intervention can be successful.

**Figure 6.1:** Proposal for the examination of stool bacteria, urine crystals and excreted oxalate content may define the risk factor for the prognosis and its recurrence rate in urolithiasis.
6.2.2 Targeted-functional Bacteria therapy may Restore the Healthy Gut Populations in Hyperoxaluria

On summarization in a ‘case-control study,’ we examined a total of 39 subjects with dysbiosis in the gut microbiota and noticed 83 % of total disease subjects were not colonized with *Oxalobacter formigenes* and also decreased active OMBS like *Lactobacillus plantarum* were explored for the first time in the human gut. In the other group ‘healthy-cohort study’ consisting of 49 healthy subjects, active OMBS abundance ranging from approximately 3 % to 18 % was reported in general population. Genus *Oxalobacter formigenes* and *Lactobacillus plantarum* are considered to be major species which help with oxalate metabolism in this population.

When we isolated *Lactobacillus plantarum* strains E2C2 and E2C5 (Lactic acid Bacteria) were analyzed for its genomic and phenotypic characterization to search for the probiotic attributes. We found promising genes which provoke to the good probiotic candidate for certain attributes. Some attributes like Bile acid tolerance, oxalate metabolism with 300 mM oxalate tolerance capacity, bile salt deconjugation ability and whereas these mentioned attributes are as a phenotypic expressions. We propose that these bacteria would be consider as one of the general probiotic candidate, shortly for the Indian population but also for the universal perspectives (Figure 6.2).
Figure 6.2: Proposal of *Lactobacillus plantarum* E2C2 and E2C5 bacterial isolates as a probiotic candidate for human consumption. Genome-level metabolic reconstruction was studied and depicted the promising health effective gene content in the isolated bacteria.

We conclude from the thesis work that the colonization of *Oxalobacter formigenes* and *Lactobacillus plantarum* in hyperoxaluria condition is associated with retention of healthy gut flora. So we can speculate that in hyperoxaluria and or even worse health situations like chronic kidney disease, ingestion of *Oxalobacter formigenes* and *Lactobacillus plantarum* would alleviate the condition. We can expect the changing ecology and favorable conditions if the artificial colonization of *Oxalobacter* and *Lactobacillus* sp. happens (Figure 6.3).

Further increased colonization with OMBS via probiotics found in natural dietary products could potentially decrease oxalates levels and improve health status in crystalluria and hyperoxaluria conditions. Further clinical and experimental studies are needed to confirm our proposals.
Figure 6.3: Illustration of hypothesis wherein Oxalobacter and Lactobacillus sp. re-colonization (or artificial colonization) in hyperoxaluria may help to oxalate clearance and healthy microbiome restoration in the human gut.