

Bacterial community divergence in groundwaters across a CKDu-endemic risk continuum in Sri Lanka

Thilakarathna PTA¹, Fareed F^{1,2}, Gunathilake R³, Danthanarayana PR⁴, Premachandra TN²,
Athukorala SN⁴, Noordeen F⁴, Wang C³, Zhang Y³, Karunaratne SHPP⁴, Jinadasa RN⁴

Introduction and Objectives: Chronic Kidney Disease of unknown etiology (CKDu) is a major public health concern, primarily affecting rural farming communities associated with the dry zone of Sri Lanka. The exact cause remains elusive. Recent studies suggest that alterations in the gut microbiome may contribute to nephrotoxicity. As local data on this association are scarce, understanding the role of microbial agents in drinking water sources in CKDu affected areas is crucial for preventive strategies. This study aimed to investigate the abundance (culturable) and composition of the bacterial community of water in CKDu endemic zones, addressing a critical gap in the existing literature.

Methods: A total of 60 groundwater samples (10x3x2) was collected from three CKDu-affected divisional secretariats, namely Padawiya, Horowpathana, and Ipalogama, representing high-risk, medium-risk, and low-risk zones of CKDu, respectively, during the dry (August 2023) and wet (January 2024) seasons. Total bacterial counts and bacterial diversity were assessed using nutrient agar (Oxoid, UK) culture-based methods and 16S rRNA gene sequencing, respectively.

Results: The culturable bacterial counts were markedly lower in the high-risk zone (6.4×10^5 CFU/mL in average) compared to medium-risk (4.8×10^8 CFU/mL in average) and low-risk (1.3×10^8 CFU/mL on average) zones, with statistically significant differences ($p < 0.050$). According to the genus-level comparisons, well water from low-risk areas showed a higher relative abundance of ubiquitous environmental genera, i.e., *Flavobacterium*, *Comamonas* and *Acinetobacter* (mean relative abundance: 35–45%), compared to high-risk areas (5–10%, $p = 0.024$). In contrast, stress-tolerant or oligotrophic genera such as *Sphingomonas*, *Methylobacterium* and *Mycobacterium* were commoner in high-risk (20–30%) than in low-risk zones (2–5%, $p = 0.041$), reflecting the selective pressure by poor groundwater quality. Species with the potential of causing nephrotoxicity via infections, toxins or altering water quality by chemical interactions, such as *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Sphingomonas*, *Mycobacterium*, were predominantly found in groundwater in CKDu-endemic areas.

Conclusions: The observed variation in microbial community structure highlights the potential of specific bacterial genera as bioindicators in groundwater linked to CKDu. These findings provide a foundation for bacterial monitoring in groundwater for CKDu risk assessments in endemic regions.

Keywords: CKDu, drinking, environmental filtering, groundwater microbiome

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¹Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka

²China-Sri Lanka Joint Research and Demonstration Centre for Water Technology, Meewathura, Peradeniya, Sri Lanka

³Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

⁴University of Peradeniya, Peradeniya, Sri Lanka

Address for correspondence: Prof R.N. Jinadasa: University of Peradeniya, Peradeniya, Sri Lanka;

Telephone: +94713045199; Email: rnjinadasa@vet.pdn.ac.lk;  <https://orcid.org/0000-0001-8538-782X>