

Antibiotic resistance profiles of *Escherichia coli* isolated from groundwaters in a CKDu-endemic region of Sri Lanka

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Introduction and Objectives: Chronic Kidney Disease of unknown etiology (CKDu) affects many people in Anuradhapura, Sri Lanka. Treating infections of these CKDu patients are challenging due to antibiotic-resistance bacteria which may originate from contaminated groundwaters. Therefore, this study aimed to identify antibiotic-resistant *Escherichia coli* in groundwaters of this affected region.

Methods: A total of 30 well water samples were collected from Padawiya, Horowpathana and Ipalogama, filtered using 0.45 µm membrane filters and cultured on m-FC (44 ± 1 °C) media for 22 ± 2 hours in triplicates to isolate faecal coliforms. All confirmed *E. coli* isolates by IMViC tests were subjected to antibiotic sensitivity testing for beta lactams (amoxicillin 30 µg), ceftriaxone 30 µg, ceftazidime 30 µg and augmentin 30 µg), aminoglycosides (gentamicin 10 µg and streptomycin 25 µg), tetracyclines (tetracycline 30 µg), fluoroquinolones (ciprofloxacin 5 µg), amphenicols (chloramphenicol 30 µg) and sulfonamide and trimethoprim combination (co-trimoxazole 25 µg), using the disc diffusion method (CLSI-2021).

Results: A total of 62 *E. coli* were isolated from these water samples. Highest resistance was observed for amoxicillin (85.5%), followed by tetracycline (56.5%), ceftazidime (50.0%) and ceftriaxone (50.0%). Notably, none of the isolates showed resistance to gentamicin. Moderate resistance was observed to co-trimoxazole (32.3%), streptomycin (19.4%), chloramphenicol (14.5%) and ciprofloxacin (17.7%). Pairwise comparisons resistance to different antibiotic classes revealed significantly higher resistance rates to beta-lactams compared to all other antibiotic classes ($p < 0.001$). Notably, tetracycline resistance was also significantly higher than resistance to aminoglycosides, amphenicols, and fluoroquinolones ($p < 0.05$), indicating class-specific resistance patterns among these isolates. The calculated Multiple Antibiotic Resistance (MAR) index, indicating resistant to three or more antibiotic classes across all isolates averaged 0.371. A total of 38 isolates (61.3%) were resistant to three or more antibiotics, with MAR indices exceeding 0.2.

Conclusion: The findings emphasize the presence of MAR *E. coli* in groundwater from Anuradhapura, Sri Lanka, with significant multidrug resistance patterns. The groundwater sources could be a risk factor for the transmission of antibiotic-resistant bacteria.

Keywords: Antibiotic Resistance; *E. coli*; Groundwaters; Multiple Antibiotic Resistance Index

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