

Detection of pathogenic *Escherichia coli*-like isolates from selected polluted water canals in Colombo district, Sri Lanka

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Introduction and Objectives: Pathogenic *Escherichia coli* in contaminated water is associated with periodic diarrhoeal epidemics, particularly in developing countries such as Sri Lanka. Hence, this study aimed to detect potential pathogenic *E. coli* strains in four selected canals in Colombo using microbiological and molecular techniques.

Methods: Two water samples each were collected from the Hamilton, Wellawatte, Dehiwala and Norris canals in Colombo and stored at room temperature, away from direct sunlight. Samples were analysed separately (not pooled) to reflect site-specific contamination. Contaminants were removed by double-filtering each sample through filter paper and then nitrocellulose membranes. Samples, including *E. coli* ATCC 25922 (positive control) and autoclaved distilled water (negative control) were cultured in duplicate on MacConkey agar. Plates were incubated at 37 °C for 24 hours. *E. coli* colonies were identified by their characteristic pink colour on MacConkey agar. Two colonies resembling *E. coli* from each plate were Gram-stained. Microscopic examination revealed the isolation of pink, rod-shaped bacteria. A single, confirmed *E. coli* colony from each plate was inoculated into Luria broth and incubated at 37 °C for 24 hours. DNA was extracted using a modified boiling cell method and visualised using the spot gel technique. Published primers were used to amplify the *eaeA* gene, and amplicons were visualised using agarose gel electrophoresis.

Results: The presence of *E. coli* in all selected canals, among other microbes, was confirmed morphologically and microscopically. Amplification of the *eaeA* gene, indicative of potential pathogenic *E. coli*-like isolates, in Norris, Wellawatte and Dehiwala canal samples was confirmed by the visualisation of bands of expected size (570 base pairs). Norris canal generated the highest frequency of positive isolates, suggesting greater contamination at the collection site. Hamilton canal isolates tested negative for the *eaeA* gene. Negative control results demonstrated no contamination.

Conclusion: The detection of the *eaeA* gene in three of the four canal samples highlights the presence of potential pathogenic *E. coli*-like microorganisms in Colombo's urban waterways. Measures should be taken to prevent public use of canal water, and enhanced monitoring strategies are needed for effective diarrhoeal disease prevention and surveillance.

Keywords: Canal, Colombo, *eaeA* gene, *E. coli*, pathogenic

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