POTENTIAL PATHOGENIC BACTERIA IN DRINKING WATER AT MIHINTALE, SRI LANKA

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Water is a fundamental human need, which is essential to sustain life. With the increasing amounts of discharged sewage, progressing urbanization, the chemicalization of agriculture and industry, water emerges as a major source of water borne bacterial pathogens, transmitting many diseases. Spread of these waterborne diseases depends on several factors such as the survival of such bacteria in water sources, infectious dose required to cause a disease, water treatments used and the season of the year.

In the North Central Province of Sri Lanka, contaminated water has become the third leading cause of death[1]. Though it has been estimated that 68.0% of the population in Mihintale have access to safe drinking water, there is no guaranteed quality assurance procedure. 10% of the people at Mihintale consume drinking water from unsafe sources[2]. *According to the surveys carried out at the Medical Research Institute of Sri Lanka, Vibrio cholerae, Salmonella typhi, Salmonella paratyphi, S. enteritidis, Escherichia coli, Campylobacter jejuni and Shigella sp., have been identified as the most common bacterial pathogens, causing water borne diseases in Sri Lanka*[3]. This study was designed to determine the bacteriological quality of tap water (delivered by the National Water Supply and Drainage Board-NWS & DB) and the well water used by the people at Mihintale Grama Niladhari Division (GND) and to enumerate the possible water borne bacterial pathogens. The study was conducted in the Mihintale GND, Mihintale Divisional Secretariat Division, Anuradhapura District of North Central province, Sri Lanka. A questionnaire guided survey was conducted with 100 households in the GND. 24 water samples (14 well waters and 10 tap waters) were collected from March 2012 to June 2012, following American Public Health Association, standards. The sample volume was 2 L. Physical parameters such as pH and temperature were analyzed as per the SLS specifications.

Water samples were analyzed for, total coliforms and fecal coliforms and for possible pathogens (Salmonella spp., *Shigella* spp., and *Vibrio* spp.). Total and fecal coliform bacteria were determined by membrane filtration technique followed by incubation of total coliforms on absorbent pads saturated with m-Endo broth (at 37 °C) and fecal coliforms on m-FC saturated pads (at 44.5 °C), for 24-48 hours. For pathogenic bacteria, Membrane Filter Technique was used followed by enrichment and selective growth. Pure cultures were identified through biochemical tests (*Bergey's Manual for Determinative Bacteriology*) and ABIS (Advanced Bacterial Identification Software). Correlation coefficient 'r' for the presence of pathogens with different bacteriological and physical parameters was determined using Karl Pearson coefficient in SPSS 10.0 software.

66% of the people in the Mihintale GND depended on tap water provided by the NWS & DB, 14% depended on water from protected wells and a 4% of the community depended on unprotected water sources. Only 22% of the community boil and filter water prior to drinking. 38% did not treat on their drinking water. This can be a serious health issue, because most of the samples examined were bacteriologically unsafe.

Mean total coliform counts obtained in both groups of water samples exceeded the World Health Organization (WHO) and Sri Lanka Standards (SLS) permissible levels, ranging from 10^3-10^5 CFU/100. 90% of tap water and 57% of well water samples exceeded the WHO and SLS permissible levels for *E. coli.*

Sixteen and 14 different cultures were isolated from tap water and well water samples respectively. In the tested tap water samples, potentially pathogenic *S. boydii.* (20.83%), *E. coli* (90.0 %) and *S. choleraesuis* subsp. *arizonae* (16.67%), *V. alginolyticus* (20.83%) and *V. hollisae* (20.83%) were identified and characterized. These pathogens are capable of causing infections of the gastrointestinal and urinary tracts. In addition to these pathogens, *Proteus* (2 spp.), *Klebsiella pneumoniae*, *Aeromonas* (3 spp.), *Pseudomonas aeruginosa*, *Edwardsiella tarda*, *Flavobacterium thalpophilum* and *Rahnella aquatilis* were identified and characterized from tap water samples. Among the bacteria isolated from well water, a prime pathogen, *V. parahaemolyticus* (4.17 %) responsible for diarrheal diseases in human, potential pathogens such as *V. damsela* (16.67%), *V. mimicus* (8.33 %), *V. metschnikovii* (25.0%), *E. coli* (57.14 %), *P. vulgaris* (54.17 %), *Aeromonas sobria* (41.67 %) and *Enterobacter sakazaki* (25.0 %) were identified and characterized. Other than these, opportunistic pathogens such as *Proteus* (2 spp.), *Enterobacter* (4 spp.), *Aeromonas* (4 spp.), *K. oxicota*, *Serratia* (4 spp.), *Providencia* (2 spp.) and *Acinetobacter* spp. were identified and characterized from well water samples. This is the first time where this range of *Vibrio* species have been reported from tap and well water sources at Mihintale GND. All of the *Vibrio* species isolated have potential to cause human infections.

pH values of all tap water samples were within the SLS permissible range, while nine well water samples (64.29%) exceeded the SLS standards (SLS 614, 1983). Correlation coefficient (r) analysis revealed that the isolation of *Vibrio* sp. was positively correlated with the indicator organisms ($r = 0.305$), water temperature ($r = 0.085$) and pH ($r = 0.217$). Also a significant correlation was observed at the 0.05 level between the isolation of *Shigella* spp. and water temperature. Therefore, prevailing dry conditions with increased water temperatures could considerably increase their concentrations. On the other hand, the isolation of *Proteus* spp. was positively correlated with the fecal coliform counts.

These gross contaminations in tap water may be due to the contaminations in the source, treatment facility, or at any other point earlier in the distribution system, indicating the possibility of formation of biofilms in the distribution pipes or in the consumer's tap. Contaminations of well waters with these pathogens might probably be attributed to the unprotected wells, drawing of water with contaminated containers, the shallowness of certain wells (5-7 m) and apparently, the lack of household hygiene that may arise from having the wells closer to the latrines. Presence of *V. parahaemolyticus* can cause diarrheal diseases in human. Presence of *S. boydii* and *S. choleraesuis* like pathogens causes gastroenteritis and systemic infections very often in immunocompromised adults and in young children. The presence of opportunistic pathogens can cause diarrhoea, bacterimia and mixed bacterial infections of extra intestinal wounds. The typical fecal pollution indicators may not access public health risk from potential pathogens, hence special monitoring programme for pathogens need to be adequately included in the water quality management. It is recommended that private wells used for drinking purposes must be tested annually at a minimum.

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