

Microbial Source Tracking Markers for Detection of Faecal Contamination in Environmental Waters

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Mini Review

Microbial source tracking (MST) describes a suite of methods and an investigative strategy for determination of faecal pollution sources in environmental waters. They rely on the association of certain fecal microorganisms with a particular host, that ranges from human [1] to agricultural animals [2,3] to pets [4] and wild animals such as gulls [5]. MST is used to appraise recreational water quality and to correlate with human health risk.

Many MST publications fixate mostly on human source contamination, as this has been an issue of concern for managers and regulators. Human sewage pollution is among the greatest concerns especially in India [7,8] for human health due to [1] the known risk of exposure to human waste and [2] the public and regulatory will to reduce sewage pollution. However, methods to identify animal sources are receiving increasing attention as our understanding of zoonotic disease potential improves.

Numerous epidemiological studies of waterborne illness in countries like India indicate that the common aetiological agents are bacteria, viruses and parasitic protozoa and the coliform presence is an important parameter for determining water pollution levels. But the presence of coliforms of soil and litter origin had raised doubt about their reliability as an indicator of the pathogen [6]. Also, the method used for coliform estimation is not precise. Hence CPCB believes that BOD is the single most reliable parameter. Based on this approach the CPCB estimated that 67% of river stretches in India is relatively clean with BOD less than 3 mg/litre. Also, poor correlations have been reported between waterborne human viruses or protozoa and thermotolerant coliforms [6,9,10]. In contrast to MST markers, faecal coliforms provided a poor metric to assess risks of exposure to faecal contamination of human origin in the rural setting in India [9]. Such a situation is critical to understand, as evident from recent drinking water outbreaks where coliform standards were met [11] and in India most of the outbreaks go unnoticed. FIB concentrations have not been well correlated with pathogens in many studies [10].

Furthermore, many epidemiology studies have failed to find a correlation between human health outcomes and FIB levels, particularly when the pollution is not from a known point source such as a wastewater treatment plant (WWTP) [14,15]. Nonetheless, water regulatory agencies have yet to come to terms with the inherent problems resulting from reliance on faecal indicator bacteria as currently determined. Fortunately, new index organisms for some pathogens look promising like *C. perfringens*, phages and viruses like Adenoviruses. As these index organisms are relatively untested worldwide, extensive trials are necessary before their general acceptance in microbial risk assessment. It should be noted that useful index organisms in one system are not necessarily of value in a different environment.

Use of multiple tests increases the surety of presence or absence of water pollution. In particular, it has been reported that at least two parameters are needed to accurately differentiate between two distinct faecal pollution sources: one specific indicator that identifies the source and another, a universal indicator that provides information on the faecal load [12]. In consequence, the indicators should be carefully selected based on appropriate statistical analyses. Persistence studies are needed to provide complementary information, addressing the effects of environmental aspects like temperature, solar radiation, salinity, pH, chemical pollutants, water filtration, turbidity, starvation, predation and presence of heavy metals, among others.

Other relevant issues are the use of methods based either on genomic targets or in the quantification of new microbial tracers through library-independent methods, the combination of methods, and comparative and integrated studies between research groups with standardised procedures to avoid differences in implementation. Also, in order to increase the validity of MST methods, it is necessary to consider temporal variability when designing the sampling scheme of the source material and constructing source libraries and increase the specificity and field

testing of DNA-based markers. The number and range of potential host sources included in MST studies must be deliberately chosen to suit the water body and particular questions associated with it [13].

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