



Closing the Door on the Fecal Coliform Assay

The fecal coliform assay, the results of which have led to numerous misinterpretations over the years, may have outlived its usefulness

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Since its inception in 1904, the fecal coliform assay has been used to assess the presence of fecal contamination in water and foods. Assays to detect *Escherichia coli*, a more specific indicator of fecal contamination, were previously not as popular due to the longer time period for detection required (five days) and their complexity. Recent advances in the detection of *E. coli*, however, have eliminated these impediments and detection occurs within 24 hours or less. Many limitations and complications have been associated with the fecal coliform assay, thereby raising questions about its continued appropriateness and usefulness in food and water testing. The microbiology literature is replete with reports of studies that correlate results of fecal coliform levels with the presence of *E. coli* including several recent examples that advocate the fecal coliform test as an acceptable indicator in manure composts and foods. However, the value of the fecal coliform assay as an indicator of fecal contamination is negated when bacteria of nonfecal origin are the principal microbes detected by the assay.

Historically, the definition of fecal coliforms has been based on methods used for their detection. Specifically, fecal coliforms are gram-negative bacilli, not sporulated, oxidase-negative, optional aerobic or anaerobic, able to multiply in the presence of bile salts or other surface agents that have equivalent properties, and are able to ferment lactose with acid and gas production in 48 h at the temperature of $44 \pm 0.5^\circ\text{C}$. Several genera of bacteria that are common contaminants of nonfecal sources (e.g., plant materials and pulp or paper mill effluents) meet this definition. Examples include *Klebsiella*, *Enterobacter*, and *Citrobacter* species. Moreover, these bacteria which are false-positive indicators of fecal contamination can grow under appropriate conditions in nonfecal niches such as water, food, and waste. The International Commission on Microbiological Specifications for Foods in its evalua-

tion of this issue reported the term fecal coliforms has arisen from attempts to find rapid, dependable methods for establishing the presence of *E. coli* and closely related variants without the need to purify cultures. Species of *Enterobacteriaceae* other than *E. coli* are associated with plants and do not indicate fecal contamination, yet they are identified as fecal coliforms by the fecal coliform assay. Hence, *E. coli* is the only valid index organism for the monitoring of foods containing fresh vegetables.

To reduce the possibility of false-positive results, a confirmatory test for *E. coli* is recommended. In spite of this precaution, there have been several instances where fecal coliform results have been incorrectly interpreted. One of the most sensational situations occurred in 1995 when the U.S. news media reported that high populations of fecal coliforms in restaurant-brewed tea indicated the presence of feces in tea. The dominant fecal coliforms identified were *Klebsiella pneumoniae* and some *Enterobacter* spp., but no *E. coli*. Although there was ample evidence of fecal coliform contamination of iced tea served in restaurants (e.g., 64% of samples at fecal coliform of $>1,100$ MPN/ml), there had been no history of outbreaks of illnesses resulting from consumption of iced tea.

Another instance where fecal coliform data have been inappropriately interpreted involved two Canadian recalls of sprouts where high levels of fecal coliforms were later identified to be *K. pneumoniae*. In the health hazard alert accompanying these recalls, a warning was issued that this organism could cause gastrointestinal illness in humans. While this bacterial strain is an opportunistic pathogen outside the intestinal tract causing respiratory and urinary tract infections, gastrointestinal illness rarely occurs. Hence, the overly cautious warning was likely due to the association of this bacterium with the fecal organism group.

A quick perusal of the Internet including both

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governmental and academic sites revealed information is being provided that fails to address the possibility that bacteria testing positive in the fecal coliform assay may originate from nonfecal sources. For example, a U.S. Environmental Protection Agency (EPA) page listing drinking water contaminants and their maximum contaminant levels stipulates that “fecal coliforms and *E. coli* only come from human and animal fecal waste.” To the contrary, as noted above, there is a preponderance of data indicating that fecal coliforms do not only originate from fecal waste. Similarly, the Kentucky Division of Water site indicates that fecal coliform bacteria “are associated only with the fecal material of warm-blooded animals” and the Food Safety Authority of Ireland site reports that “faecal coliforms found in water are a direct indication that the water has been contaminated with animal or human effluent.” Collegiate and K-12 academic sites also provide similar misleading information. Unfortunately, these generalizations can lead to misinterpretation of results by those who do not have a complete understanding of the fecal coliform assay and the subtleties associated with interpreting the results of such assays.

Concerns regarding the inappropriate interpretation of results of the fecal coliform assay and its limited usefulness as an indicator of fecal contamination are not new. They have surfaced several times over the past decade. When the issue of fecal coliforms in tea made media headlines, it was suggested that the fecal coliform assay be reevaluated for its usefulness in food testing. The following year, two commentaries published in *ASM News* opined that the fecal coliform term should be excluded from microbiology. This was further supported by investigators of a study comparing *E. coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency, who concluded that *E. coli*-based effluent and stream standards (not fecal coliform standards) should be developed to protect public health. A subsequent review of the suitability of the coliform group as an indicator of microbial water safety led other investigators to recommend elimination of the fecal coliform assay. This proposal was further corroborated by studies revealing that only 50% of fecal coliform colonies enumerated as fecal coliforms in foods were identified as *E. coli*.

In the past few years, several changes in monitoring protocols have already been initiated by national and international regulatory agencies. In the European community as well as in Australia and New Zealand, the “fecal coliforms” term has been replaced by what is considered a more appropriate descriptor of this class of microorganisms, “thermotolerant coliforms”. Both WHO’s Guidelines for Drinking Water Quality and the Australian Drinking Water Guidelines, however, continue to advocate that thermotolerant coliform measurements are an acceptable alternative to *E. coli* measurements. While this change in terminology reduces the likelihood that positive results may be interpreted as meaning the presence of fecal contamination, it does not eliminate the possibility that nonfecal coliforms may be present and give positive results.

In 1986, the U.S. EPA published a document that encouraged states to use *E. coli* or enterococci as the basis of their water quality criteria to protect fresh recreational waters and to use enterococci as the basis for water quality criteria in marine waters. While these guidelines have been criticized, a systematic review and meta-analysis of data reaffirmed these recommendations. More specifically, this analysis revealed that *E. coli* was a more consistent predictor of gastrointestinal illness than other bacterial indicators in fresh water. Despite these recommendations, state and local authorities have been slow to respond in adopting these guidelines. To address some of the advantages and impediments to implementation of these guidelines, costs for the three bacterial indicators were surveyed in the Tacoma/Seattle region and were found to be fairly comparable and thus not a limiting factor. In contrast, an inherent weakness cited by the Washington State Department of Ecology was that using enterococci as an indicator organism in marine waters would complicate efforts to model data obtained from freshwater sources in which *E. coli* was monitored. Another weakness is the continuing requirement by the Food and Drug Administration to use fecal coliforms as an indicator microorganism in shellfish marketed across state borders. Despite this requirement, no significant relationship has been observed between levels of *E. coli* and enterococci and non-*E. coli* fecal coliforms in oysters. Consequently, the continued use of fecal coliforms as an indicator in shellfish would likely hinder widespread acceptance of more appropriate indicators. Moreover, in a National Academies of Science (NAS) report to evaluate candidate indicator organisms and/or indicator approaches, the committee was adverse to abandoning the current indicator microbes until new and better methods are developed and validated. While the NAS Committee foresaw the advent of increasingly sophisticated and powerful molecular biology techniques that would provide new opportunities for the development of improved assays for indicator microbes, we contend that immediate replacement of the fecal coliform assay with an *E. coli* assay would apply the best science available to providing public health protection.

In conclusion, physicians and public health officials have repeatedly misinterpreted results of the fecal coliform assay when applied to food, beverage, or water samples. To prevent future occurrences, the fecal coliform assay should at a minimum be redefined to specifically qualify that it is not a reliable indicator of either *E. coli* or the presence of fecal contamination. An even better alternative would be to eliminate the fecal coliform assay as an indicator of fecal contamination of foods, beverages, and water. The *E. coli* assay is a more reliable indicator of fecal contamination, although not absolute, and could serve as a replacement for the fecal coliform assay.

Literature citations and relevant references which provide the basis for this commentary can be found in the online version of this article.