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# Use of indicator bacteria for assessment of water: change of a paradigm?

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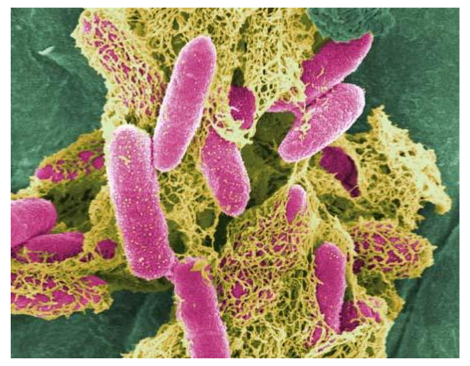


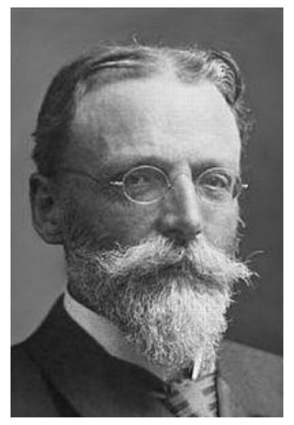
*Eschericia coli*

## Introduction

The concept of **fecal indicator bacteria (FIB)** in determining the sanitary quality of water was first proposed in the 1880's when workers began to use bacteriologic media to assess microbial presence in water and food commodities (Ashbolt et al.,2001). Although alternative bacterial indicators have been suggested (e.g., **fecal streptococci, sulphite-reducing Clostridia**), various forms of **coliform bacteria** have been used since the 1920's as standard microbial indicators for quality assessments. The cultivation and enumeration of coliform indicator bacteria (e.g., **total coliforms**, **fecal coliforms**, and **Escherichia coli** (E. coli)) remains the primary method for testing the biologic quality of fresh waters for drinking, recreation, fishing, and industry (Farnleitner et al., 2000). Currently, culturally-based methods of screening are preferred over non-culturally/genetics based tests (O'Brien, 2011).

FIB predict the potential presence and risks of pathogenic microbes in water as they circumvent the need to specifically test for select pathogens transferred via the fecal-oral route. FIB are characterized as: nonpathogenic, easy to detect and count, exhibiting survival characteristics similar to the pathogens they indicate, and showing positive correlation with pathogenic microbes affecting man.

The concept of indicator bacteria for the assessment of water pollution is still being employed today and new methods for detecting the presence of *E. coli* are being explored daily. Biochemical techniques like polymerase chain reaction (PCR) amplification and genetic hybridizations have been investigated recently and some researchers suggest that these methods are better able to monitor the surveillance of *E. coli* and select pathogens, like *Salmonella spp.*, in waterways.



**Theodor Escherich**
**(namesake for *E. coli*)**

### Indicator Organisms and Water Quality Assessment: a Chronological History

1880 – **Von Fritsch** – observed *Klebsiella spp*. in human feces
1885 – **Percy and Grace Frankland** – first routine examinations of fecal bacteria in water
1885 – **Theodor Escherich** – discovered *Bacillus coli* within feces of warm-blooded animals
1899 – **Klein and Houston** – suggested the use of *Clostridium perfringens* as indicator bacteria
1901 – **Horrocks** – suggested the term 'coliform bacteria' for those bacteria resembling *B. coli*
1902 – **Winslow and Hunnewell** – suggested fecal streptococci to be used as pollution indicator organisms
1904 – **Eijkman** – suggested E. coli to be imperative for bacterial assays due to its heat tolerance and fermentative ability
1907 – **Winslow and Walker** – reported *B. coli* more commonly of fecal origin vs other coliform species
1919 – **Castellani and Chalmers** – renamed *B. coli* as *Escherichia coli* in honor of Escherich
1948 – **Mackenzie et al.** – distinguished 'fecal coliform' from 'coliform' through thermotolerance and positive Indole reaction
1969 – **Geldreich and Kenner** – suggest fecal coliform/fecal strep (FC/FS) ratio for source tracking bacterial pollution
1978 – **Cabelli** – Clostridium perfringens found in less than 35% of human source
1986 – **USEPA** – recommends E. coli as the better indicator for assays of fresh water; enterococci for brackish and marine waters
1991 – **Gauthier et al.** – new definition for *E. coli* to include lack of urease and possessing β-glucuronidase
1994 – **HMSO** – new definition for total coliform bacteria to include species of Family Enterobacteriaceae, possessing β- galactosidase, and growth at 37° C
1997 – **WHO** – report on various species of Enterococcus and Streptococcus to be included as fecal strep
2000 – **Dore et al**. – propose the use of F+ coliphage (FRNA bacteriophage) as indicators for enteric viruses in water
2005 – **Harwood et al**. – suggest the use of multiple indicator organisms to sufficiently monitor reclaimed water

Source: Ashbolt, N.J., Grabow, W.O.K., and Snozzi, M. 2001. Indicators of microbial quality. In: *Water Quality: Guidelines, Standards, and Health.* L. Fewtrell and J. Bartram, Eds., IWA Publ., London.

## Pros and cons of indicator bacteria in the recent water quality literature

### Indicator bacteria vs Pathogenic bacterial species

**Pros**

-Significant correlation with *Campylobacter* (Hörman et al., 2004)

-Fecal indicators were surrogates of pathogenic bacteria in almost all samples of an agricultural setting (Wilkes et al., 2009)

-Levels of *E. coli* and enterococci correlated positively with levels of *Campylobacter* in rainwater harvest systems (Ahmed et al., 2011)

-Indicator bacteria found in higher concentrations in ag animals hence, are better predictors for pathogens associated with human infection (Cox et al., 2005)

-*E. coli* is the best indicator of bovine pollution in New Zealand (Sinton et al., 2007)

**Cons**

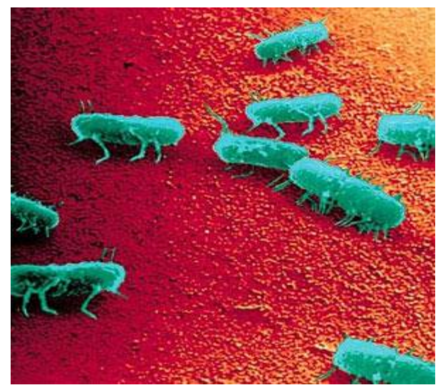
-Not a good indicator for Shiga toxin microbes (Smith et.al., 2009)

-No relation to pathogenic bacteria in oysters (De Paola et al., 2010)

- Low correlation with thermotolerant *Campylobacter* (St. Pierre et al., 2009)

-Indicator bacteria cling to and persist in periphyton in environmental waters (Ksoll et al., 2007)

-*E. coli* can survive extended periods in temperate soils and re-contaminate waters (Ishii et al., 2007)



***Salmonella***

**Campylobacter**

### Indicator bacteria vs pathogenic viruses

**Pros**

-Significant correlation to HAV and enterovirus presence in recreational marine waters (Gersberg et al., 2006)

-Significant correlation with norovirus in surface water (Hörman et al., 2004)

-Indicator bacteria correlated positively with pathogenic viral entities in subtropical marine waters (Abdelzaher et al., 2010)

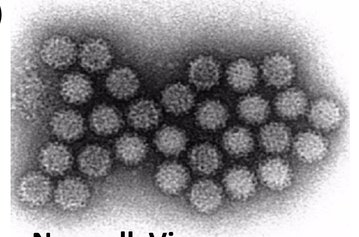
-*E. coli* shows significant correlation with viral pathogen in surface waters of N. Europe (Horman et al., 2004)

**Cons**

-No significant correlation between RV (rotavirus) presence and fecal coliform levels (He et al., 2011)

-lack of correlation to presence of specific viruses (HAV and NOV) in oysters (De Paola et al., 2010)

-Not an effective indicator for enteric viruses (Fong and Lipp, 2005)



**Norwalk Virus**

### Bacterial indicators vs protozoan parasites

**Pros**

-Positive correlation between distribution of thermophilic amoebae and fecal indicators (Delattre et al., 1991)

-Significant correlation with *Giardia* and *Cryptosporidium* in surface water (Hörman et al., 2004)

-Indicator bacteria correlated positively with pathogenic protozoan entities in subtropical marine waters (Abdelzaher et al., 2010)

-Presence of *E. coli* shows significan correlation with Protozoan in surface waters of N. Europe (Hörman et al., 2004)

-Coliform bacteria and enterococci "acceptable" as indicators of poor environmental water quality in Europe (Schets et al., 2008)

**Cons**

-No clear relationship between fecal coliform levels and detection of *Cryptosporidium* and *Giardia* (Wohlesen et al., 2006)

**Giardia**

## Discussion

FIB have been used since the early 20th Century as the standard reference for microbial pollution/contamination of water and foodstuffs and, for that period of time, they proved to be "good" indicators of microbial presence. 100 years later, workers still report high, positive correlation between counts of FIB and counts of many bacterial, viral, and protozoan pathogens in a wide variety of environments. Yet many workers have reported no/low correlations of FIB to water borne pathogens (see Pros/Con boxes), while others suggest screening for more than one indicator for increased reliability in situations where public health is directly affected .

Over this time, methods of direct microbial testing have become more precise in selectivity and quantification. Multiple tube fermentation (MTF) was the first technique used to test for the presence and estimation of FIB. Broth tubes were eventually replaced by more accurate and less costly membrane filtration (MF) procedures in the 1950's, requiring less time and labor. Since the early 1990's, defined substrate tests (DST)/enzyme analyses have shown improved enumeration accuracy while requiring less lab time and no confirmatory testing. DST for select bacterial pathogens are being developed.

**Results from our lab:**
Throughout late Spring and early Fall 2011, we examined levels of coliform bacteria, *E. coli*, and species of *Salmonella* within samples taken from three streams in the vicinity of Prince Edward County, VA. Comparisons of the counts obtained for *E. coli* and *Salmonella* are presented below in Figures 1 and 2. Figure 1 compares counts of *E. coli* and *Salmonella* within one of the study streams. Figure 2 shows the correlation between matched counts of *E. coli* and *Salmonella* taken from individual stream samples. Bacterial counts were transformed in Figure 2 to Log10 values to reveal this relationship.
Although the raw counts of *E. coli* do not correlate well with counts of *Salmonella*, a correlation between Log 10 transformed counts of these bacteria reveals a positive and significant (P<0.05) relationship.

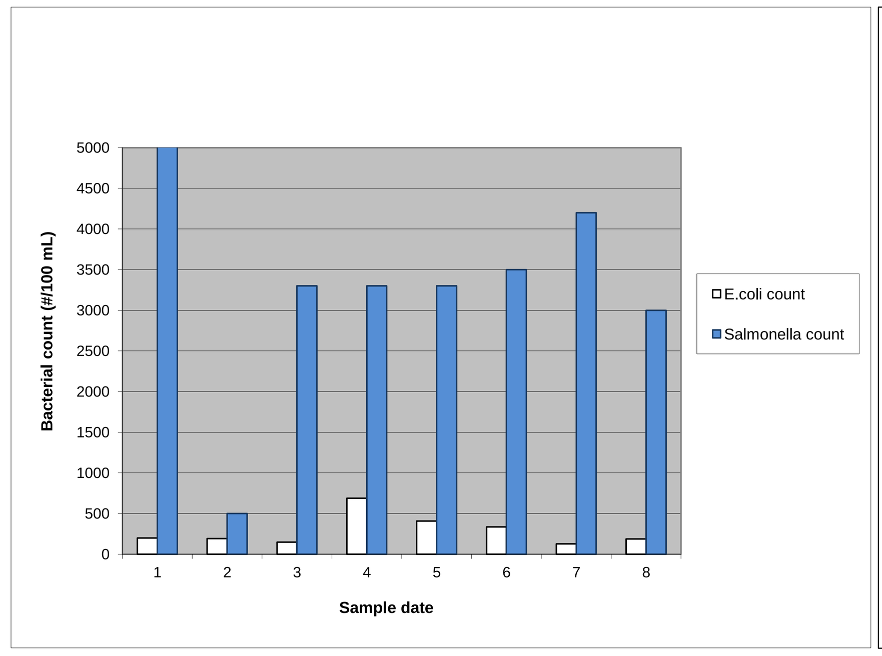



**Figure 1.** ***Sayler's Creek: E. coli vs Salmonella count per sample date***

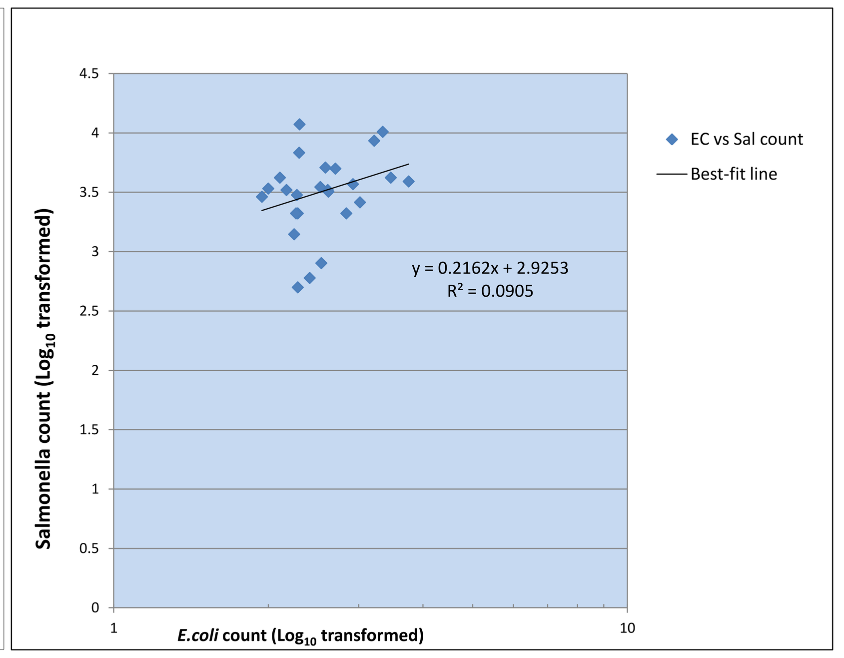



**Figure 2. Pooled and Log10 transformed *E. coli and Salmonella counts***

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