

### MYTH # 3

## **Coliforms Are Non-pathogenic Indicators of Sewage Fecal Pollution in Food, Water and Sludge**

### A short History Lesson

### Disease Causing Organisms in Your Food and Water Courtesy of EPA

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Help for Sewage Victims

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For several years, I have wondered about the public claims by experts that:

- 1) the presence of coliform only indicate the possibility that disease causing organisms may be present in food or water;
- 2) fecal coliform is a better indication of animal and human fecal contamination; and
- 3) the presence of E. coli confirmed animal and human fecal contamination.

My research has lead me to confirm, based on biology text books, studies, and government documents, that the experts have either been lying to us or have no idea that the claims are based on 30 primary pathogenic members of the Enterobacteriaceae family. Bacteriologists have split the Enterobacteriaceae family into three groups based on temperature:

- 1) E.coli is the primary member with optimum growth at 37°C (98.6°F);
- 2) those that have optimum growth at 35°C (95°F) are called coliform;
- 3) those with thermotolerant (heat resistant) genes that only show some minor biological activity at 44.5-46°C (114.8°F) are called fecal coliform.

### **A One Hundred Year Old Myth Promoted By EPA, FDA and USDA**

For the past forty odd years it has been assumed that Congress, federal agencies , supporting scientists, and engineers knew what they were doing in their effort to promote the use of pathogen contaminated sewage sludge (and recycled water) on fruits and vegetables under a 1981 policy based on Christiaan Eijkmans's elevated temperature fermentation test for heat resistant coliform bacteria. This has resulted in foodborne illnesses showing a dramatic increase from 2 million infections annually in 1986 to 76 million infections in 1999. According to Centers for Disease Control (CDC), there were only a small percentage (325,000 ) of the 76 million infections that caused hospitalization and and even smaller percentage (5,000) that resulted in deaths. CDC changed its approach in 2010. Based on nine disease causing organisms it estimates that there were only 48 million illnesses, 128,000 hospitalizations and 3,000 deaths annually from foodborne illnesses.

The story is different for methicillin-resistant Staphylococcus aureus (MRSA) which does not show up in the standard food and water tests for the family Enterobacteriaceae (i.e., coliform and fecal coliform). Hospital stays for MRSA increased from 1,900 in 1993 when EPA released its current Part 503 sludge policy to 368,800 in 2005 with over 18,000 deaths. Many of those most affected are children and the elderly with little chance of proving cause and effect unless the agencies identify a specific organism in a water or food outbreak. They are also the major drain on medical facilities and cost the economy most when they become victims.

Heat resistant fecal coliform indicators are a few of the Enterobacteriaceae members that show some biological activity at 44.5°C (112.1°F). According to the pretreatment standards in 40 CFR 403.5(b)(5), Heat well below that temperature will inhibit biological activity in a POTW resulting in Interference,

and industry is prohibited from releasing wastewater into a POTW where the Treatment Plant exceeds 40°C (104°F).

Coliform are all Enterobacteriaceae with optimum growth about 95°F. This group includes many disease causing organisms in the primary families of: *E. coli*; *Citrobacter*; *Enterobacter*; *Klebsiella*; *Shigella*; *Salmonella*; and *Yersinia* as well as secondary families of *Averyella*; *Budvicia aquatica*; *Buttiauxella noackiae*; *Calymmatobacterium*; *Cedecea*; *Edwardsiella*; *Ewingella*; *Hafnia alvei*; *Kluyvera*; *Koserella*; *Leclercia adecarboxylata*; *Leminorella*; *Moellerella wisconsensis*; *Morganella*; *Pantoea*; *Photobacterium*; *Proteus*; *Providencia*; *Rahnella aquatilis*; *Serratia*; *Tatumella*; *Xenorhabdus*; and *Yokenella regensburgei*. These bacteria caused 40% of the 1.7 million health care acquired infections in 2002 which resulted in 98,987 deaths. Causes of death for the 98,987 were 35,967 for pneumonia, 30,665 for bloodstream infections, 13,088 for urinary tract infections, 8,205 for surgical site infections, and 11,062 for infections of other sites. With the exception of surgical infections, the rest of the infections could be acquired in rural settings through sewage sludge /biosolids contaminated food, soil and water.

The use of the Celsius temperature is confusing to many people. Therefore, for clarity, Celsius will be converted to Fahrenheit in this article. As a further aid, more quotes are included than would be allowed by the style guides.

The government's 1981 policy was based on Nobel Prize winner in Physiology or Medicine for 1929, Christiaan Eijkman's, 1904 glucose broth fermentation test for *Bacillus Coli* (*Escherichia coli*) at 114.8°F. Eijkman created the myth when he convinced scientists that only *E. coli* from humans and other warm blooded animals could show some growth in water within 48 hours at the elevated temperature to indicate fecal contamination. Today, we know there is no scientific basis for assuming that *E. coli* found at 114.8°F are only from humans and other warm blooded animals since most animals or humans would be dead by the time their core temperature reached that elevated level. As an example, according to EPA, medical examiners generally list heat exposure as a primary or contributing cause of death if the core body temperature exceeds 105°F.

[http://www.epa.gov/heatisland/about/pdf/EHEguide\\_final.pdf](http://www.epa.gov/heatisland/about/pdf/EHEguide_final.pdf)

Part of Eijkman's problem was that little was known about disease causing organisms at that time, their health effects or how to test for them in food or water. In the intervening years the tests were refined for bacteria that could be used as fecal contamination indicators. *E. coli* and other members of the Enterobacteriaceae family that ferments lactose to produce acid and gas within 48 hours were chosen. Bacteriologists then split the Enterobacteriaceae family of pathogens into three separate test groups for testing food and water: 1) Enterobacteriaceae with optimum growth at about 35°C (95°F), just below the human body temperature of 37°C (98.6°F), were claimed to be from cold blooded animals, plant, soil, and water with no sanitary significance and called coliforms; 2) Enterobacteriaceae with thermotolerant (heat resistant) genes that only show some minor biological activity at 46° (114.8°F) were claimed to be only from humans and other warm blooded animals and called fecal coliform; and 3) *E. coli*, the primary member of the Enterobacteriaceae family with optimum growth at 37°C (98.6°F), is said to confirm fecal contamination.

The myth is reflected in the U.S. Food and Drug Administration (FDA) fecal contamination tests for food and water where the temperature is slightly lower than the original Eijkman test. According to

FDA, “Fecal coliform analyses are done at 113.9°F for food testing, except for water, shellfish and shellfish harvest water analyses, which use 112.1°F.” The Environmental Protection Agency (EPA) also uses the temperature of 112.1°F to assess fecal contamination in water. Any evidence of heat resistant bacteria at these temperatures would cause food or water to fail the tests. On the other hand, it has little to do with human disease causing organisms since medical laboratories test for pathogenic Enterobacteriaceae at 98.6°F.

While these test temperatures do little to protect public health from contaminated food or water, Congress created new food safety laws in 2010 giving FDA more power. However, in June 2011, House Republicans, argued that the U.S. food supply is 99 percent safe, cut millions of dollars from FDA's budget, effectively blocking implementation of the food safety laws.

The most unusual use of the fecal coliform test is its use to assure the safety of sewage sludge / biosolids promoted as a fertilizer on grazing land, food crops, forests, parks, school grounds, home lawns and gardens. While E. coli will double every 20 minutes at 98.6°F, the elevated temperature test actually restricts the growth of E. coli colonies. Not only that but the test time was lowered from 48 to 24 hours which also dramatically lowers the colony count. For grazing land, food crops fiber crops and forests with a 30 day restriction, EPA allows approximately two million counted heat resistant colonies of Enterobacteriaceae per gram of Class B sludge / biosolids at the end of the test period. For unlabeled unrestricted use on parks, school grounds, home lawns and gardens EPA allows approximately one thousand counted heat resistant colonies of Enterobacteriaceae per gram of Class A sludge / biosolids at the end of the test period. No one has any idea how many heat resistant bacteria are in each colony, nor how many non-heat resistant disease causing organisms are in the waste.

It is apparent that after 100 years some scientists researching food, water and sludge are still confused by the tests. An example of this confusion can be found in Cornell's 2004 final report, “Hygienic Implications of Small-Scale Composting in New York State” where they write the human gut temperature is 113°F. While the actual core temperature of the body is 98.6°F, the report states, “Fecal coliform are a sub-group of total coliforms. Total coliform counts often include organisms that do not reside in the intestinal tract, so methods have been developed to test for fecal coliforms, which by definition are supposed to be coliform microbes that grow when a temperature of 113°F (i.e., the temperature of the human gut) is maintained during incubation.”

. <http://cwmi.css.cornell.edu/coldcompost.pdf>

## **Background**

Most people have heard of E. coli as well as the mythical coliforms and fecal coliforms as indicators of sewage contamination, but have no idea that the terms only refer to E. coli and similar coli-like-forms of the pathogenic gram-negative Enterobacteriaceae family incubated at different temperatures. The myth is still promoted by EPA, FDA and USDA for water and food testing as well as testing sludge / biosolids because the agencies know that 99% of the exposed individual's immune systems are sufficient to prevent a serious disease from any of the major pathogenic organisms in sewage contaminated foods.

Since the cause of many drinking water and food outbreaks are never identified, the German physician and bacteriologist Robert Koch's four postulates are put forth as liability protection. Koch made these

observations in 1890 based on his germ theory of disease:

1. The organism must be found in abundance in all individuals suffering from the disease, but should not be found in healthy individuals.
2. The organism must be isolated from a diseased individual and grown in pure culture.
3. The cultured organism should cause disease when introduced into a healthy individual.
4. The organism must be reisolated from the inoculated individual and be identical to the original organism.

The following are some exceptions to the four postulates recognized by Koch and modern scientists.

1. *Some healthy people and animals are carriers of deadly disease causing organisms. Typhoid Mary is an example.. Another is antibiotic resistant bacteria found in healthy individuals.*
2. *Some organisms have yet to be identified and others such as Mycobacteria tuberculosis, prions, and viruses can not be grown in pure culture. Still others are viable but nonculturable by standard laboratory methods.*
3. *Some individuals have genetic immunity, some develop immunity early in life and others have a good but imperfect immune system.*
4. *In 1928, British Medical Officer Frederick Griffith discovered the potential for recombinant DNA to occur in an individual using two strains of Streptococcus pneumoniae. A pathogenic smooth strain with a polysaccharide capsule was heat killed and could not cause disease alone. This was matched with a rough nonpathogenic strain that did not cause pneumonia. When both were injected in mice at the same time the mice died. When Griffith reisolated the organism he found it had picked up the polysaccharide capsule from the heat killed organism.*

The need for a human fecal indicator appeared to be evident after Dr. John Snow demonstrated in 1854 that exposure to human fecal material in drinking water was very dangerous and deadly when he discovered the cause of the great London Vibrio cholera outbreak was sewage. The outbreak was stopped when he removed the pump handle from a public drinking water well. The indicators used are Enterobacteria coliforms, fecal coliforms, E. coli, as well as streptococci and Enterococci.

Today, we know that all of these indicators are at a minimum opportunistic pathogens for those individuals with compromised immune systems. Since indicators only require low levels of disinfectants, it doesn't tell us anything about disease causing organisms that require high levels of disinfectants such as spore forming bacteria or viruses like Adenoviruses, that cause gastrointestinal tract infections, conjunctiva, central nervous system damage, urinary tract infections, malignancy and human obesity or the cancer causing Papillomasvirus, said to be sexually transmitted, which has infected 25 million U.S. children and young women. If these disease causing organisms are in the water they may be transmitted through the sexual organs without any sexual contact. Since they are also known to be in treated sewage used to irrigate or fertilize food crops they may be contacted through the

food chain without any sexual contact. According to EPA documents, Viruses may survive on plants for up to 2 months and in soil for up to 1 year.

In a 1981 federal sludge policy document, "Land Application of Municipal Sewage Sludge For The Production of Fruits and Vegetables, A Statement of Federal Policy and Guidance", signed by EPA, FDA, and USDA, no agency claimed responsibility for protection the public's health. However, all three Agencies agreed that heavy metals, toxic organic compounds, and pathogenic microorganisms are a great concern. In the policy statement, EPA, FDA and USDA state that the safety of food grown on sludge is assured as long as the guidance is followed. Moreover, there is a caveat that the "government can not offer any indemnity against product recall, seizure, or other enforcement actions, -- However, the risk of such enforcement actions would be no greater than the risks associated with normal farming and processing practice." The basic guide was in the 1979 solid waste regulation Part 257. The caveat was necessary because EPA, FDA and USDA, "recognizes that pathogen survival is greater in warm, moist environments, than in extremely arid or colder environments."  
<http://thewatchers.us/EPA/1981-policy-EPA-USDA-FDA.pdf>

Government documents show food poisoning incidences increased dramatically after EPA released its new proposed sludge policy regulation in 1989.

1986 there were one to two million cases of food poisoning (Gerba) (EPA Risk Assessment for landfilling sludge)

1990 there were about 6 million cases of food poisoning

1994 there were about 33 million cases of food poisoning 9,000 deaths EPA-USDA- CDC-Report to President -  
From Farm to Table (1997)

1996 there were about 80 million cases of food poisoning (Ralph J. Touche-Chief Sanitarian -Public Health Service

1997 there we about 81 million cases of food poisoning (GAO-report)

1998 CDC estimates 360 million cases of acute diarrhea, Most from unknown source of exposure.  
(1987 estimate) 9,100 deaths annually.

1999 (Mead,et.al) (CDC) estimates there are only about 76 million foodborne cases annually, 325,000 Hospitalized and 5,000 deaths. CDC used these figures until 2010, 6.3 million illnesses per month, 27,000 people hospitalized each month, 416 dead each month.

In 2010, CDC released the following statistics, 48 million foodborne illnesses, 128,000 hospitalizations and 3,000 deaths each year in the U.S. However, the numbers are only based on foodborne infections caused by nine disease causing organisms: 1) four indicator coliforms, Salmonella, one strain of Escherichia coli (STEC) O157, Shigella and Yersinia; 2) three non-coliforms, Campylobacter, Listeria and Vibrio; and 3) two protozoa parasites, Cryptosporidium and Cyclospora. CDC acknowledges that three disease causing organisms are not counted, norovirus, Clostridium perfringens, and Toxoplasma, because tests are generally not available to them in clinical labs.

CDC states,

Most foodborne infections cause diarrheal illness, ranging from mild to severe. Also, persons in susceptible populations and some healthy persons can develop severe complications, such as hemorrhagic colitis, bloodstream infection, meningitis, joint infection, kidney failure, paralysis, miscarriage, and other problems. Beyond their health effects, foodborne illnesses can cause emotional and economic hardship; for example, Salmonella alone causes approximately 1 million foodborne infections (2) and costs \$365 million in direct medical expenditures\* annually (7), and the societal cost of a single fatal case of Escherichia coli (STEC) O157

infection has been estimated at \$7 million  
[http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6022a5.htm?s\\_cid=mm6022a5\\_w](http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6022a5.htm?s_cid=mm6022a5_w)

### **Tests with no meaning**

The implication of federal and state policies for sewage sludge / biosolids promoted as a fertilizer and sewage effluent used for irrigation is that even though some disease causing organisms may be in the treated sewage, their use is safe because users and the public are protected by the policies. Before we get into the regulatory tests for recycled sewage effluent and sludge /biosolids spread on food crops and drinking water, we need to review CDC's Guidelines for Biosafety Laboratory Competency – Guiding Principles:

No regulation or guideline can ensure safe practices. Individual and organizational attitudes regarding safety will influence all aspects of safe practice, including willingness to report concerns, response to incidents, and communication of risk. Each organization should strive to develop a culture of safety that is open and nonpunitive, encourages questions, and is willing to be self-critical. Persons and organizations must be committed to safety, be aware of risks, behave in ways that enhance safety, and be adaptable. Scientists understand that practices should be refined as observations are made, hypotheses tested, findings published, and technical progress achieved. The same holds true for safety in the laboratory, which should evolve as experience is gained and as laboratory activities change. As laboratorians gain more knowledge over time concerning how to recognize and control hazards, the level of risk that is considered acceptable should become smaller, with the goal of moving continuously to eliminate or reduce risk to the lowest reasonably achievable level.

[http://www.cdc.gov/mmwr/preview/mmwrhtml/su6002a1.htm?s\\_cid=su6002a1\\_w](http://www.cdc.gov/mmwr/preview/mmwrhtml/su6002a1.htm?s_cid=su6002a1_w)

When you start researching information on sewage contamination indicators, it gets confusing due to the early assumptions that coliform have no sanitary significance. As an example: drinking water testing starts with the coliform test. If that test is positive, a fecal coliform test is performed. To verify fecal contamination from humans, an E. coli test is performed. If no E. coli is found, the drinking water is considered to be pure. Descriptions in government documents and scientific studies are as confusing as a group of blind people describing an elephant. Since they give no indication that this elephant could cause serious illness or even kill you, we have to wonder if they know what they are dealing with or simply covering up the danger to the public to prevent a national panic.

According to EPA's Drinking Water documents, these indicators such as E. coli are generally not harmful, meaning of course, they are generally not harmful to people with good immune systems. Moreover, there is the implication that E. coli will only grow at the elevated temperature of the fecal coliform test. Furthermore, these documents fail to acknowledge that E. coli has been documented in pristine soil and water environments. Not only that, but scientists have been deliberately inserting antibiotic resistant genes into genetically modified E. coli bacteria for over thirty years to confirm a successful transfer of DNA. Many of the genetic experiments were dumped into the sewers where DNA transfer between bacteria have been reported since Mark Meckes 1981 study on UV light disinfection of sludge. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC241834/>

The myth in these documents state:

Members of two bacteria groups, coliforms and fecal streptococci, are used as indicators of

possible sewage contamination because they are commonly found in human and animal feces. Although they are generally not harmful themselves, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems.

Fecal coliforms, a subset of total coliform bacteria, are more fecal-specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper mill wastes.

*E. coli* is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals.

Fecal streptococci generally occur in the digestive systems of humans and other warm-blooded animals.

Enterococci are a subgroup within the fecal streptococcus group. Enterococci are distinguished by their ability to survive in salt water, and in this respect they more closely mimic many pathogens than do the other indicators.

<http://water.epa.gov/type/rsl/monitoring/vms511.cfm>

There appears to be confusion between what EPA Region 7 says in a May 26, 2011 news release and the drinking water documents. Region 7 stated *E. coli* is an indicator of fecal coliform bacteria in the Mississippi River flood water, while the drinking water documents above state that *E. coli* is “a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals.” Due to the major difference between coliform and *E. coli* numbers, EPA's results and explanation indicate it used 95°F for testing coliform and 112.1°F for testing *E. coli*/ fecal coliform. MPN means EPA counted colonies at the end of the test and assumed each colony represented the most probable number at the beginning of the test.

Region 7 states:

Results from the sampling show nine samples of receding floodwaters had total coliform levels ranging from 5,475 to 15,531 mpn/100ml (most probable number of colony forming units per 100 milliliters of water). However, analysis also showed *E. coli* bacteria levels, which are an indicator of fecal coliform bacteria in the floodwaters, ranged from 0 to 38 mpn/100ml, which are well below Missouri's official standard of 126 mpn/100ml for swimming and 1,134 mpn/100ml for fishing, wading and boating activities. Harmful bacteria in the water can cause symptoms such as stomach ache, fever, vomiting and diarrhea. Persons exposed to fecal coliform can become ill if they have an open cut, wound or scrape that comes into contact with contaminated water. Symptoms include fever, redness and swelling at the site of an open wound. If these symptoms occur, a doctor should be consulted.

<http://thewatchers.us/coliform-v-ecoli-comparison.html>

*E. coli* survives much better on land than in the water. We discovered this to be true when doing a soil sample test on the Alice Minter Trust farm in 1998. We suspected that pathogens were draining off the 1,200 acre Kansas City, Missouri's sludge farm. While we didn't understand the full implication at that time, it demonstrated the difference between the coliforms *Salmonella* and *E. coli* tested at 95°F and

the heat resistant fecal coliforms Salmonella, E. coli, etc. tested at 112.1°F. I didn't know at the time the numbers represented actual colonies counted at the end of the test. Still, the dry weight results were disturbing because it showed the fecal coliform test indicated only a minor percentage of the coliform pathogens growing in sludge, food or water:

Sample 1: Fecal coliform -- 9,000 MPN colonies per 100 milliliters (incubated at 112.1°F)  
Salmonella -- >800,000 MPN colonies per 100 milliliters (incubated at 95°F)

**Sample 2: Fecal coliform -- 3,000 MPN colonies per 100 milliliters (incubated at 112.1°F)**  
E. coli -- >800,000 MPN colonies per 100 milliliters (incubated at 95°F)

Sample 4: Fecal coliform -- 50,000 MPN colonies per 100 milliliters (incubated at 112.1°F)  
Strep -- 100,000 MPN colonies per 100 milliliters (incubated at 95°F)

<http://thewatchers.us/pathogens/test-comparison.html>

Based on the fecal coliform tests, according to EPA, the soil would be perfectly safe for growing food crops for human consumption. However, based on the E. coli, Salmonella and Streptococcus levels the farm was a biohazard site where crops could not be safely grown. I didn't want to be responsible for anyone's death and shut down the farm operation. These tests are enlightening because the sewage sludge department of EPA claims that Class B sewage sludge is a safe biosolids for grazing land and food crops as long as it has less than 2 million MPN heat resistant fecal coliforms per gram (one milliliter) dry weight and there is a 30 day restriction. Since Class B sludge / biosolids are mostly liquid and only heat resistant colonies are counted on the outside of the sludge biofilm at the end of the test and reported as colony forming units, no one has any idea what type or how many types of pathogens are spread on agricultural land in each liter (one thousand milliliters/grams) of sewage waste. Moreover, EPA and its partners claim Class A sludge is absolutely safe to handle with no restriction if it only contains 1,000 MPN of heat resistant colony forming units of fecal coliform per gram (milliliters dry) weight.

The most damning part of the myth is that while the high temperature of the test inhibits the growth of all members of Enterobacteriaceae except a few heat resistant strains of E. coli, Citrobacter, Enterobacter, Klebsiella, and Salmonella these same bacteria are doubling about every 20 minutes in the sludge / biosolids headed for farms, parks, school grounds as well as your lawn and garden.

Breaking the prevailing myths in environmental testing starts with recognizing the fact that E. coli is not a species of fecal coliform, nor is it a species of coliform. Instead it is the primary species identified in the Enterobacteriaceae family of coli-like-forms of bacteria that produce gas and acid in the fermentation test when incubated at just below the normal body temperature at 95°F. for 24 hours. Fecal coliform are the members that have picked up a heat resistant gene from thermophilic bacteria.

Most members of the Enterobacteriaceae family used as indicators are pathogenic when they escape the intestinal tract and infect the blood or some other organ. Most of those same bacteria will show no activity in the fecal coli fermentation test at 112.1°F because they grow best at 98.6°F. Yet, the early assumption that coli-like-forms of bacteria normally present in the intestinal tract are not pathogens is still prevalent, even though science has proven that was/is a false assumption.

## The Indicators

Until recently, scientists have either trivialized infectious biological agents, or have caused confusion by using the term, etiologic agents, rather than discussing disease causing organisms. They have called them bugs, germs, plaque, biofilm, microorganisms, pathogens, coliform and fecal coliform. Animal diseases that can infect humans are called zoonoses.

To understand the lack of science behind the myth we need to look at each indicator test. As EPA states in the Drinking Water Contaminant list, total coliforms (including fecal coliform and *E. coli*) are “Not a health threat in itself; it is used to indicate whether other potentially harmful bacteria may be present.” The coliform tests are a very inexpensive test for the Enterobacteriaceae family. EPA has approved ten enzyme based tests for coliform and *E. coli* in water since 2002. Those tests are designed to suppress bacteria other than members of the Enterobacteriaceae, especially the pathogen *Aeromonas*.

Industry regulators, scientists and environmental engineers had rather not discuss the fact that the indicators only require low levels of chemicals, heat or cold to inactivate, while many of the major disease causing organisms require high levels of disinfection. EPA's Part 503 sludge policy only requires that some members of the Enterobacteriaceae family be inactivated to some degree. Most of this inactivation is due to the high temperature test parameters of 112.1°F for fecal coliform. The lack of moisture or food can also cause inactivation. However, when you bring everything back to normal, the bacteria are reactivated. An example is that ATCC laboratory samples are shipped freeze dried and reactivated by the receiving laboratory. Of course most people remember the 2001 dried powdered Anthrax bioterrorism incident which killed 5 people.

According to the ATCC website:

ATCC is a private, nonprofit biological resource center (BRC) and research organization whose mission focuses on the acquisition, authentication, production, preservation, development and distribution of standard reference microorganisms, cell lines and other materials for research in the life sciences.

### **E. coli Indicator:**

According to EPA:

**E. coli is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals.**

This is not necessarily true according to the World Health Organization. It states:

The range of non-faecal bacteria represented in the coliform group and the environmental growth of thermophilic (faecal) coliforms *Klebsiella* spp. and *E. coli* (Ashbolt et al. 1997; Camper et al. 1991) have concerned bacteriologists and sanitary engineers since the 1930s (Committee on Water Supply 1930).

[http://www.who.int/water\\_sanitation\\_health/dwq/iwachap13.pdf](http://www.who.int/water_sanitation_health/dwq/iwachap13.pdf)

While the uninhabited Krakatau Islands were sterilized in the 1883 volcanic eruption, one hundred years later 27 types of antibiotic resistant soil bacteria, including *E. coli*, were found. *E. coli* has been engineered to produce ethanol and biopharmaceuticals as well as used to remediate soil contaminated

with oil, pesticides, etc. The primary laboratory strain of *E. coli* is culture strain K-12 which was isolated from a patient in 1922. It has been cultured so long it has lost the capability to be pathogenic. However, there are thousands of strains of enteroaggregative *E. coli* (EAEC) (largest outbreak in food – Germany), enterohemorrhagic *E. coli* (EHEC) (largest outbreak in water– Canada) , enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), and Uropathogenic *E. coli*, UPEC) that are pathogenic.

According to FDA, “*Escherichia coli*, originally known as *Bacterium coli commune*, was identified in 1885 by the German pediatrician, Theodor Escherich. *E. coli* is a member of the family Enterobacteriaceae, which includes many genera, including known pathogens such as *Salmonella*, *Shigella*, and *Yersinia*. Although most strains of *E. coli* are not regarded as pathogens, they can be opportunistic pathogens that cause infections in immunocompromised hosts. There are also pathogenic strains of *E. coli* that when ingested, cause gastrointestinal illness in healthy humans.”\ <http://www.fda.gov/food/scienceresearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/cm064948.htm>

EPA, FDA and USDA are gambling with our life by deliberately exposing us to superbugs like *E. coli*. There is little doubt that any bacteria that can kill you is a superbug. *E. coli* is no exception, medical studies show *E. coli* strains cause inflammatory diarrhea , destruction of red blood cells, HUS kidney failure (hemolytic-uremic syndrome), urinary tract infections, bacteremia, meningitis, severe, lung infection, pneumonia, abscesses in the lining of the lungs (empyema), necrotizing "flesh eating" infections in the urinary tract as well as the abdominal cavity and death. Many strains are antibiotic resistant. The most unusual aspect is that the use of antibiotics will increase the HUS rate of death.

According to biological textbooks, *E. coli* was first isolated in hospitals as a cause of infant diarrhea in 1935, thirty-one years after Eijkman developed the fermentation test for heat resistant *E. coli* which he assumed was only fecal coliform. Actually, the diarrhea outbreak occurred during the winter of 1933-34 at the Memphis General Hospital. *E. coli* was found in the heart, blood, spleen, ileum, jejunum, colon, and middle ears of infants. There was a forty-seven per cent infant death rate. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1559361/pdf/amjphnation00908-0075.pdf>

In a 1900 English study of 57 cases of puerperal infection (child-bed fever), thirty-one infections proved fatal due to *E. coli*, *Staphylococcus* and *Streptococcus*. In 1915, the British Medical Journal reported on a case of acute septic meningitis due to *E. coli* following a skull wound. By 1950, the family Enterobacteriaceae was an established cause of urinary tract infections. One of the first cases of antibiotic resistant *E. coli* was reported in 1954 in a bone abscess. The first documented case of enterohemorrhagic *Escherichia coli* O157:H7 was an Oakland, California naval officer in 1975. This was followed in 1982 by outbreaks in Michigan and Oregon caused by McDonald hamburgers. The first infection in England and Wales was reported in 1982, and the first case in Scotland in 1984. By September of 2006 there were 3,520 unique *E. coli* O157:H7 strains reported to CDC PulseNet since 1996 (JAMA. 2006;296(18):2195-2196).

In the 1903 paper, “The Pathogenicity of *B. coli* in Relation to the Bacteriological Examination of Water”, WG Savage explained the scientific confusion, when he said, “*B. coli* are looked for in water not because they themselves are harmful, actually or potentially, but because they are indicators of contamination.” He went on to say, “The question of virulent *B. coli* can therefore be narrowed down to

the following: - Does the fact that an isolated B. coli is pathogenic, when obtained from a water, indicate that the contamination is harmful and of necessity dangerous, and does the fact of its being non-virulent indicate freedom from dangerous pollution? Such a conclusion can be by no means maintained.” Savage also pointed out the inconsistent results obtained at different incubation temperatures. “In this particular sample B. coli was found in 40 c.c., but not in smaller amounts, while the numbers of organisms per c.c. [gram] were 3 colonies at 98.6°F., and 145 colonies at 71.6°F.”

Most regulators, industry scientists and environmental engineers continue to ignore the fact that the higher the test temperature, the less bacteria you find in the tests. Savage appears to be the first to show the large colony count drop due to test temperatures (e.g., 145 colonies per gram at 71.6° degree Fahrenheit versus 3 colonies per gram at 98.6° Fahrenheit).

Perhaps more disturbing, Savage found that 1/3 of the B. coli samples from pure and contaminated sources were virulent. Some strains would only cause a temporary illness, while some could kill a guinea-pig in as little as 17 hours. He also found that nonpathogenic B. coli that had passed through 3 mice could then kill the 4<sup>th</sup> mouse, and yet, leave the guinea-pig unaffected. In effect, the B. coli test was of little use in trying to determine the quality of water.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2235995/pdf/jhyg00314-0093.pdf>

In the book, “Modern Surgery, General and Operative (1919)”, the editors noted E. coli could be found in the mouth, nose, vagina, on the skin, and under the nails as well as in water considered to be pure. They noted that while it was ordinarily harmless, it could acquire pathogenic power and enter the blood system where:

This bacillus may be responsible for appendicitis, peritonitis, inflammation of the genito-urinary tract, pneumonia, inflammation of the intestine, leptomeningitis, perineal abscess, cholangitis, cholecystitis, myelitis, puerperal fever, wound infections and septicemia. It is the cause of many abscesses about the intestine and responsible for many ischio-rectal abscess. From the pus of an appendicitis abscess we may perhaps obtain culture of Escherich's bacillus, but usually find also streptococci or staphylococci, and sometimes pneumococci. Colon bacilli introduced into the system by tainted food may be responsible for epidemic pneumonia. A few years ago there was such an epidemic in Middlesbrough, England (Oliver, in "Brit. Med. Jour." April 30, 1910)

<http://www.archive.org/details/modernsurgerye02costgoog>

Daniel D. Jackson, laboratory Division, Department of Water Supply, Gas and Electricity, New York City, tried to stop the myth in 1910. He warned scientists through the Journal of the American Public Health Association:

"The term B. coli as an indication of fecal contamination in water and milk has been so often misapplied that the result has been much confusion and frequent misinterpretation of bacterial examinations. It has been the custom of many bacteriologists to throw out of sanitary consideration all bacteria which do not absolutely conform to the so-called "typical" B. coli. There are many known varieties, all of fecal origin and closely related to typical B. coli, which will be described in this paper, and there probably exist many more varieties which will be discovered in the future. Any of these varieties, when they occur in water or milk, have a sanitary significance, and because of their close relationship, all should be included in the B.

coli group."

The group consists of four species:

B. communiior, (Durham.)

B. communis, (Escherich.) [E. coli]

B. aerogenes, (Escherich.) [Aerobacter aerogenes, Enterobacter aerogenes, klebsiella aerogenes]

B. acidi-lactici, (Hueppe.)

A study of this classification shows that thirteen out of seventeen known varieties of B. coli have been isolated from feces or diseased conditions; and that seven of these varieties have been isolated from water. Of the seven varieties isolated from water, four would conform to so-called "typical" B. coli, in spite of the fact that they are here grouped under three distinct species, B. communiior, B. communis, and B. acidi-lactici. It is evident that the so-called typical B. coli does not exist as such, but that the entire group is typical of fecal contamination when water or milk examinations are to be considered.

<http://thewatchers.us/EPA/13/1911-classification-B-coli-group.pdf>

In Max Levine's 1920 study, State University of Iowa, he suggested a special media was needed to study the relative incidence of B. coli and B. aerogenes in water and feces. The purpose of the study was to find a media that would inhibit the growth of one while allowing the other to flourish. Levine noted the maximum rate for multiplication of B. coli is at about 98.6°F., while B. aerogenes grow best at about 86° Fahrenheit. Since then some strains of Aerobacter, Enterobacter, and Klebsiella have been identified as aerogenes. <http://thewatchers.us/EPA/2/1921--E-coli-Enterbactor.pdf>

By 1931, Fred O. Tonney and Ralph E. Noble. Bureau of Laboratories and Research, Department of Health, Chicago, had joined the debate on the sanitary significance of B. aerogenes in the paper "The Relative Persistence of Bact. Coli and Bact. Aerogenes in Nature". Their research had shown that B. coli was found at a ratio of 100 to 1 in feces whereas in nature the ratio was 1 in 20. They also found that B. aerogenes was more persistent and grew better in the natural environment of tree stumps than B. coli. However, over the length of the experiments both survived for long periods of time with little real difference. The major difference in survival time was caused by seasonal factors. When samples were deposited in the spring when sludge is usually spread, both survived for over 170 days and up to 228 days. <http://thewatchers.us/EPA/2/1931-presistence-E-coli-Enterbactor.pdf>

In the 1977 book, "Bacterial Indicators / Health Hazards Associated with Water". A.W.Halley and Bernard J. Dutka, Eds, American Society for Testing and Materials, say that over 90 percent of bacteria in raw sewage is E. coli. However, in the treatment plant, E. coli only makes up about a third to a fourth of the bacteria because the other 3/4 are 25% Klebsiella and 50% Enterobacter-Citrobacter.

[http://books.google.com/books?](http://books.google.com/books?hl=en&lr=&id=GCWL5nlC7TEC&oi=fnd&pg=PA48&dq=fecal+coliform+influent&ots=AuSkWrBgkh&sig=)

[hl=en&lr=&id=GCWL5nlC7TEC&oi=fnd&pg=PA48&dq=fecal+coliform+influent&ots=AuSkWrBgkh&sig=](http://books.google.com/books?hl=en&lr=&id=GCWL5nlC7TEC&oi=fnd&pg=PA48&dq=fecal+coliform+influent&ots=AuSkWrBgkh&sig=)

### **Shiga toxin (Verocytotoxin)-producing Escherichia coli in the community**

E. coli 0157:H7 is a mutant containing a toxin gene from a 12 year pandemic outbreak of Central

American shigella starting in 1968 that killed over 12,500 people the first year in Guatemala alone. In 1969-70 the Central American Shigella Dysentery strain caused a smaller diarrhea outbreak in California. Apparently, the Shigella toxin gene was used in a recombinant DNA experiment with E. coli to create chimeric bacteria never before seen in nature. While this may have been part of Herbert Boyer and Stanley Cohen's experiments at Stanford leading up to the 1974 genetic recombinant patent application, but it was more likely a product of the Naval Biosciences Laboratory at Oakland, California. E. coli 0157:H7 was first documented in a naval officer in Oakland in 1975. Seven years later it made the jump to cattle (1982), where it showed up in McDonald's hamburgers in Oregon and Michigan. It also spread to England and Wales in 1982 and then jumped to Scotland in 1984. It was better known for the 1993 Jack-in-the-Box outbreak until it showed up in wild boars for the first time in 2006, near a contaminated spinach field irrigated with recycled sewage water known to, and allowed to, contain some levels of unknown bacteria by state law.

According to the Oklahoma Department of Health, the coliforms enterohemorrhagic E. coli, Shigella, Salmonella and Yersinia as well as the non-coliform Campylobacter may cause Hemolytic uremic syndrome (HUS). This disease destroys red blood cells and causes kidney failure. In the past it primarily affected children. However, that is no longer the case. Moreover, according to the Department of Health, not all cases of HUS are caused by these shiga toxin producing bacteria. "while uncommon, a variety of viral and bacterial infections, as well as some cancers, can cause HUS" Hospital cost for a HUS victim is about \$100,000.00. Between 8% of HUS victims treated without antibiotics and 50% treated with antibiotics die.

[http://www.ok.gov/health/Disease\\_Prevention\\_Preparedness/Acute\\_Disease\\_Service/Disease\\_Information/Hemolytic\\_Uremic\\_Syndrome.html](http://www.ok.gov/health/Disease_Prevention_Preparedness/Acute_Disease_Service/Disease_Information/Hemolytic_Uremic_Syndrome.html)

The largest outbreak to date was the 2000 Walkerton, Canada drinking water E. coli 0157:H7 outbreak that killed seven people and sickened 2,500 others in the rural mid-western town of 5,000. The outbreaks get bigger and the bacteria get more deadly.

A new even more deadly strain, E. coli O104:H4, has been found in a foodborne outbreak in Germany that causes an extremely high rate of HUS and neurological problems. It attacks healthy young adults with over 3,820 confirmed sick, 864 with hemolytic uremic syndrome and 44 dead as of 23 June 2011. One expert stated one hundred will need transplants or dialysis for the rest of their life. This strain of Enterohemorrhagic Escherichia coli (EHEC) has not been found in cattle. Most scientists, including CDC, claim cattle are the host reservoir for these deadly pathogens. According to an article in the April – June 1998 edition of CDC's "Emerging Infectious Diseases" (Volume 4, Number 2) by James P. Nataro et al., University of Maryland School of Medicine, Baltimore, "first described in 1987 – Enterohemorrhagic Escherichia coli (EHEC), an increasingly recognized cause of diarrhea in children in developing countries, has been particularly associated with persistent diarrhea (more than 14 days), a major cause of illness and death." CDC does not list this type in its summary of **Shiga toxin-producing E. coli** E. coli. However, in its page on non-**Shiga toxin-producing E. coli** it states, "Enterohemorrhagic E. coli probably cause chronic diarrhea in HIV-infected patients."

## **CDC Summary of Diarrheagenic Escherichia coli (non-Shiga toxin-producing E. coli) 2005**

### **Etiologic Agent**

Escherichia coli of many different serotypes, categorized into four major groups according to

virulence mechanisms: enterotoxigenic (ETEC); enteropathogenic (EPEC); enteroinvasive (EIEC); and enteroaggregative (EAagg EC). Other groups (e.g., diffusely adherent *E. coli*) are less well established as pathogens.

### **Incidence Unknown;**

very few laboratories can identify these organisms. Enterotoxigenic *E. coli* are the most common cause of travelers' diarrhea and have caused several foodborne outbreaks in the United States. There are an estimated 79,420 cases of ETEC in the United States each year. EPEC and EIEC primarily infect children in the developing world. Enterotoxigenic *E. coli* probably cause chronic diarrhea in HIV-infected patients.

### **Sequelae**

Sequelae of ETEC, EPEC, and EIEC infection are not well described. Enterotoxigenic *E. coli* may cause chronic diarrhea.

[http://www.cdc.gov/ncidod/dbmd/diseaseinfo/diarrecoli\\_t.htm](http://www.cdc.gov/ncidod/dbmd/diseaseinfo/diarrecoli_t.htm)

### **CDC's Summary of Shiga toxin (Verocytotoxin)-producing Escherichia coli:**

2009 edition of the Biosafety in Microbiological and Biomedical Laboratories

*Escherichia coli* is one of five species in the gram-negative genus *Escherichia*. This organism is a common inhabitant of the bowel flora of healthy humans and other mammals and is one of the most intensively studied prokaryotes. An extensive serotyping system has been developed for *E. coli* based the O (somatic) and H (flagellar) antigens expressed by these organisms. Certain pathogenic clones of *E. coli* may cause urinary tract infections, bacteremia, meningitis, and diarrheal disease in humans, and these clones are associated with specific serotypes.

The diarrheagenic *E. coli* strains have been characterized into at least four basic pathogenicity groups: Shiga toxin (Verocytotoxin)-producing *E. coli* (a subset of which are referred to as enterohemorrhagic *E. coli*), enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and enteroinvasive *E. coli*. In addition to clinical significance, *E. coli* strains are commonly-used hosts for cloning experiments and other genetic manipulations in the laboratory. This summary statement provides recommendations for safe manipulation of Shiga toxin-producing *E. coli* strains. Procedures for safely handling laboratory derivatives of *E. coli* or other pathotypes of *E. coli* should be based upon a thorough risk assessment.

### **Occupational Infections**

Shiga toxin-producing *E. coli* strains, including strains of serotype O157:H7, are a demonstrated hazard to laboratory personnel. The infectious dose is estimated to be low—similar to that reported for *Shigella* spp., 10-100 organisms. Domestic farm animals (particularly bovines) are significant reservoirs of the organisms; however, experimentally infected small animals are also sources of infection in the laboratory. Verocytotoxin-producing *Escherichia coli* have also been in wild birds and rodents in close proximity to farms.

### **Natural Modes of Infection;**

Cattle represent the most common natural reservoir of Shiga-toxin producing *E. coli*. Transmission usually occurs by ingestion of contaminated food, including raw milk, fruits, vegetables, and particularly ground beef. Human-to-human transmission has been

observed in families, day care centers, and custodial institutions. Water-borne transmission has been reported from outbreaks

#### **Agent Summary Statements;**

Bacterial Agents associated with swimming in a crowded lake and drinking unchlorinated municipal water. In a small proportion of patients (usually children) infected with these organisms, the disease progresses to hemolytic uremic syndrome or death.

#### **Laboratory Safety and Containment Recommendations;**

Shiga toxin-producing E. coli are usually isolated from feces. However, a variety of food specimens contaminated with the organisms including uncooked ground beef, unpasteurized dairy products and contaminated produce may present laboratory hazards. This agent may be found in blood or urine specimens from infected humans or animals. Accidental ingestion is the primary laboratory hazard. The importance of aerosol exposure is not known.

Strict compliance with **BSL-2 practices**, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Procedures with aerosol or high splash potential should be conducted with primary containment equipment or in devices such as a BSC or safety centrifuge cups. Personal protective equipment, such as splash shields, face protection, gowns, and gloves should be used in accordance with a risk assessment. The importance of proper gloving techniques and frequent and thorough hand washing is emphasized. Care in manipulating faucet handles to prevent contamination of cleaned hands or the use of sinks equipped with remote water control devices, such as foot pedals, is highly recommended. Special attention to the timely and appropriate decontamination of work surfaces, including potentially contaminated equipment and laboratory fixtures, is strongly advised. ABSL-2 practices and facilities are recommended for activities with experimentally or naturally infected animals.

#### **Special Issues**

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

<http://www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf>

No one wants to talk about Shiga-toxin producing E. coli in drinking water, except scientists from outside the United States. In a July 2006, P. W. Ramteke and Suman Tewari, Deemed University, India, found 45 (78.9%) type-able strains of heat resistant (fecal) E. coli from 188 drinking water sources, of which 15 (26.3%) were pathogenic serotypes. The 15 heat resistant/thermotolerant/fecal E. coli in drinking water were in groups 04 (Uropathogenic E. coli, UPEC), 025 (Enterotoxigenic E. coli, ETEC), 086 (Enteropathogenic E. coli, EPEC), 0103 (Shiga-toxin producing E. coli, STEC), 0157 (Shiga-toxin producing E. coli, STEC), 08 (Enterotoxigenic E. coli, ETEC) and 0113 (Shiga-toxin producing E. coli, STEC).

There is also a myth being promoted that agricultural use of drugs is responsible for the rise in antibiotic resistant bacteria. Scientists have known for over sixty years that members of the Family

Enterobacteriaceae transfer antibiotic resistant DNA between themselves. Lederburg and Tatum, Yale University, reported on finding mutant bacteria resistant to x-radiation, ultraviolet light, and nitrogen mustard gas in 1946 and gene recombination in *E. coli* in 1947. As noted earlier, EPA's Marks Meckes found UV light increased the percentage of antibiotic resistant bacteria during sewage treatment in 1981. Bernard Davis, Public Health Service reported on penicillin resistant mutants used to create rare substances in 1948. Herbert Boyer and Stanley Cohen filed the patent in 1974 for process to create recombinant bacteria by inserting alien DNA into the bacteria. Antibiotic resistant genes were also inserted to confirm transfer had taken place.

## **COLIFORM INDICATORS**

According to EPA's Drinking Water document:

Coliforms are used as indicators of possible sewage contamination because they are commonly found in human and animal feces. Although they are generally not harmful themselves, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems.

When we review the coliform, it sure looks like there a lot of pathogens such as *E. coli*; *Citrobacter*; *Enterobacter*; *Klebsiella*; *Shigella*; *Salmonella*; and *Yersinia* as well as secondary families of *Averyella*; *Budvicia aquatica*; *Buttiauxella noackiae*; *Calymmatobacterium*; *Cedecea*; *Edwardsiella*; *Ewingella*; *Hafnia alvei*; *Kluyvera*; *Koserella*; *Leclercia adecarboxylata*; *Leminorella*; *Moellerella wisconsensis*; *Morganella*; *Pantoea*; *Photorhabdus*; *Proteus*; *Providencia*; *Rahnella aquatilis*; *Serratia*; *Tatumella*; *Xenorhabdus*; and *Yokenella regensburgei*.

EPA's 1986 Total Coliform Rule attempts to make light of finding these pathogenic gram negative bacteria in water. According to EPA, "Coliforms are a group of bacteria, most of which are harmless. At first glance, it might seem strange that a harmless group of bacteria such as coliforms could cause such commotion [cause a boil water order]. But like police tape and chalk outlines, coliform bacteria are often found at the scene of a crime even though they are not themselves criminals. -- The presence of coliform bacteria in tap water suggests that the treatment system is not working properly or that there is a problem in the distribution system that moves treated water from the treatment plant to customer homes."

Coliform are animal, plant, soil and water bacteria in the Enterobacteriaceae family many of which cause disease in humans. In retrospect, one strain of *Yersinia*, the coliform causing the Black Plague was estimated to have killed between 75 and 100 million people between the years 1340 and 1350. It is still around today and very treatable. Medical experts at the time attributed the plague outbreak to bad air, God's anger and/or the Jews. Today, we blame the victims for not washing their hands or failing to follow safety recommendations in food preparation.

In the late 1800s, during an outbreak in China, the host of *Yersinia* was determined to be fleas, with rats as the primary host. During the 19<sup>th</sup> and 20<sup>th</sup> century, a primary pathogen in sludge on EPA's 1989 part 503 Sludge Regulation list, *Vibrio cholera*, created large scale pandemics. Unfortunately, *Vibrio cholera* does not show up in the coliform test.

The original coliform test was adopted by the Public Health Service in 1914 to evaluate potential fecal

contamination of water by *Bacillus coli* (*E. coli*). The test was later expanded to coli-like-forms of bacteria using a combination of four isolation methods, Indole, Methyl Red, Voges Proskauer and Citrate utilization collectively known as IMViC test. Total coliforms (including *E. coli*) isolated by this method is the working name for a relative small class of gram-negative bacteria that ferment lactose to produce gas and acid when incubated at 35 degree C for 24-48 hours. The coliform bacteria group include animal, human, plant, soil and water bacteria that are pathogenic to humans and fall into the current family Enterobacteriaceae.

In the 2001 World Health Organization (WHO) document, “Water Quality: Guidelines, Standards and Health”, Editors Lorna Fewtrell and Jamie Bartram, stated:

The concept of ‘coliform’ bacteria, those bacteria resembling *B. coli* [*E. coli*], was in use in Britain in 1901 (Horrocks 1901). Hence, the total coliforms can best be described as a range of bacteria in the family Enterobacteriaceae varying with the changing composition of the media. The use of bacteria as indicators of the sanitary quality of water probably dates back to 1880 when Von Fritsch described *Klebsiella pneumoniae* and *K. rhinoscleromatis* as micro-organisms characteristically found in human faeces (Geldreich 1978).

**Faecal indicator:** A group of organisms that indicates the presence of faecal contamination, such as the bacterial groups thermotolerant coliforms or *E. coli*. Hence, they only infer that pathogens may be present.

The range of non-faecal bacteria represented in the coliform group and the environmental growth of thermophilic (faecal) coliforms *Klebsiella* spp. and *E. coli* (Ashbolt et al. 1997; Camper et al. 1991) have concerned bacteriologists and sanitary engineers since the 1930s (Committee on Water Supply 1930).

[http://www.who.int/water\\_sanitation\\_health/dwq/iwachap13.pdf](http://www.who.int/water_sanitation_health/dwq/iwachap13.pdf)

In 2001, EPA adopted the Coliphages presence/absence Method 1601 for coliforms in ground water and drinking water based on male-specific (F+) RNA or DNA viruses and somatic DNA viruses. EPA states, “Coliphages are viruses (bacteriophages) that infect *E. coli* and are indicators of fecal contamination.”

The most interesting thing about the test is the potential for exposure to biohazards from coliforms in sewage. EPA warns:

This method is not intended for use in **biosolids samples** or as a test for microorganisms other than coliphage. – The biohazards and the risk of infection by pathogens associated with handling raw sewage are high in this method. – Samples may contain high concentrations of biohazardous agents and must be handled with gloves. Any positive reference materials also must be handled with gloves in an appropriate laboratory hood. The analyst must never place gloves near the face after exposure to media known or suspected to contain pathogenic microorganisms. Laboratory personnel must change gloves after handling raw sewage or any other items which may carry pathogenic microorganisms.

<http://www.epa.gov/microbes/1601ap01.pdf>

The Food and Drug Administration (FDA) was a signatory to the 1981 EPA Federal Policy (Now Part 503 Regulation) to use pathogen contaminated sewage sludge on fruits and vegetables as a fertilizer.

FDA acknowledges the coliform family of Enterobacteriaceae includes known pathogens such as E. coli, Salmonella, Shigella, and Yersinia as well as enteric bacteria like Citrobacter, Klebsiella and Enterobacter, which also happen to be pathogens. They are part of a group of Gram-negative, facultative anaerobic rod-shaped bacteria that ferments lactose to produce acid and gas within 48 hours at 95°F.

<http://www.fda.gov/food/scienceresearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/cm064948.htm>

**Representative sample of disease for the six coliforms from medical studies:**

E. coli strains cause inflammatory diarrhea, destruction of red blood cells, HUS kidney failure (hemolytic-uremic syndrome), urinary tract infections, bacteremia, meningitis, severe lung infection, pneumonia, abscesses in the lining of the lungs (empyema), necrotizing "flesh eating" infections in the urinary tract as well as the abdominal cavity and death. Many strains are antibiotic resistant. The most unusual aspect is that the use of antibiotics will increase the HUS rate of death.

Salmonella cause arterial infections or endocarditis, pneumonia or empyema, urinary tract infections, meningitis, septic arthritis and osteomyelitis, typhoid / enteric fever (rose spots) on the chest and abdomen, intracranial, bone and joint, soft tissue, infections of pancreatitis, gallbladder, liver and kidneys (glomerulitis), genitourinary tract infections, necrotizing fasciitis "flesh eating" infections, bacteremia, pneumonia, heart valve infection, pericarditis, peritonitis, otitis media, cholecystitis, endophthalmitis, cutaneous abscesses, cephalhematoma, and death. Many strains are antibiotic resistant and produce poisonous Hydrogen Sulfide (H<sub>2</sub>S) gas.

Shigella strains cause watery diarrhea, seizures, eye inflammation and reactive arthritis (Reiter's syndrome), and necrotizing "flesh eating" enterocolitis infections. Intestinal perforation may occur and part of the rectum may be pushed out of the body causing permanent loss of bowel control. Infections may lead to death. Many strains are antimicrobial resistant and produce poisonous Hydrogen Sulfide (H<sub>2</sub>S) gas.

Yersinia cause severe abscess of the lung, diarrhea, hepatic and splenic, focal (oral) infections, bacteremia, pharyngitis, meningitis, osteomyelitis, pyomyositis, conjunctivitis, pneumonia, acute proliferative glomerulonephritis, peritonitis, and primary cutaneous, necrotizing "flesh eating" enterocolitis infection, pseudotuberculosis, acute gastroenteritis and mesenteric lymphadenitis, arthritis, septicemia, and erythema nodosum, Reiter's syndrome, Black Plague and death. Some strains are antibiotic resistant and produce poisonous Hydrogen Sulfide (H<sub>2</sub>S) gas.

Citrobacter cause infections of the urinary tract and infant meningitis as well as necrotizing meningo-encephalitis (25 – 50% death rate). It is multiple antibiotic resistant and produces poisonous Hydrogen Sulfide (H<sub>2</sub>S) gas.

Enterobacter cause hospital infections, urinary tract and respiratory tract infections, necrotizing enterocolitis "flesh eating" infections, and death. Many strains are antibiotic resistant and produce poisonous Hydrogen Sulfide (H<sub>2</sub>S) gas.

Klebsiella cause urinary tract infection, pneumonia, lung destruction, surgical wound infections, blood infections known as bacteremia/septicemia, which may progress to shock and death if not treated early in an aggressive fashion, and necrotizing fasciitis, "flesh eating" infections. Many strains are antibiotic resistance and produce poisonous Hydrogen Sulfide (H<sub>2</sub>S) gas.

[http://thewatchers.us/1\\_index-bacteria.html](http://thewatchers.us/1_index-bacteria.html)

As you can see the total coliform bacteria group includes some very deadly pathogenic organisms in the family Enterobacteriaceae. Yet, in the 1989 proposed part 503 Sludge Regulation, EPA listed only three coliform as primary pathogens, E. coli, Salmonella and Shigella. Only two non-coliform bacteria were listed as primary pathogens by EPA: Campylobacter and Vibrio Cholerae.

While it will not show up in the coliform or fecal coliform test, Campylobacter grows at little above the fecal coliform temperature at 113°F. It is the most common cause of bacterial foodborne illness and is found in chicken, turkey, duck, goose, game fowl, unpasteurized (raw) milk, undercooked meats such as beef, pork, lamb, shellfish, produce, and eggs. Campylobacter infections commonly cause diarrhea and bacteremia, with consequent endocarditis, osteomyelitis, or septic arthritis, Guillain-Barré syndrome, necrotizing colitis infection and death. Vibrio Cholerae is the causative agent of gastroenteritis, cholera, septicemic shock, wound infections, necrotizing fasciitis and death

Today, it seems scientists still don't want to talk about the full range of disease causing coliforms. As an example, according to the Southern Illinois University at Carbondale's course outline for "Introduction to Medical Microbiology" there are only six coliform of concern. It states, "coliform community acquired infections are caused by: Escherichia, Enterobacter, Klebsiella, Serratia, Citrobacter and Proteus. "E. coli accounts for 85% of urethrocystitis cases, 80% of chronic bacterial prostatitis cases and 90% of acute pyelonephritis cases. Proteus, Klebsiella and Enterobacter may produce urinary tract infections. Proteus may also be responsible for some renal infection stones, due to the production of the enzyme urease and subsequent alkalization and supersaturation of urine. In addition, K. pneumonia is responsible for approximately 3% of bacterial pneumonia cases and is more severe than that produced by S. pneumoniae. E. coli can also produce several different types of diarrheal disease."

<http://www.cehs.siu.edu/fix/medmicro/colif.htm>

The outline neglects to mention:

Proteus cause diarrhea of infants, wound and urinary tract infections, cystitis, secondary invader in various localized suppurative pathologic processes, and death. It is antibiotic resistant and produces poisonous Hydrogen Sulfide (H<sub>2</sub>S) gas.

Serratia cause urinary tract infections, wound infections, endocarditis, osteomyelitis, pneumonia, necrotizing fasciitis "flesh eating" infection as well as death. It is also antibiotic resistant.

While the term coliform is seldom used by doctors anymore, forty percent of all healthcare-associated infections involve coliforms. In effect, 60% of the healthcare-associated disease causing organisms would be missed in the coliform test. According to Ayesha Mirza, MD Assistant Professor, Pediatric Infectious Diseases, University of Florida College of Medicine Jacksonville, "Healthcare-associated infections result in excess length of stay, mortality and healthcare costs. In 2002, an estimated 1.7

million healthcare-associated infections occurred in the United States, resulting in 99,000 deaths. In March 2009, the CDC released a report estimating overall annual direct medical costs of healthcare-associated infections that ranged from \$28-45 billion.” <http://emedicine.medscape.com/article/967022-overview#a0199>

While most sewage and water experts, like EPA, claim coliforms don't cause disease, the District of Columbia Water and Sewer Authority (DCWSA) warns, “EPA allows up to five percent of the samples collected in a month to be positive for total coliform – immune compromised people are at a greater risk in developing illness. DC Water encourages immune compromised individuals to consult with their doctor regarding extra precautions to avoid infection.” In the last 12 months DCWSA reports coliform was only found in a small percentage of tests during the December and February testing cycles. This was a major improvement over the June 2008 - June 2009 report which showed coliform was found during 8 of the 12 testing periods. The implication is that immune compromised people who drink water in Washington DC should consult their doctor concerning the disease causing organisms that are too expensive to test for or may not be culturable by standard laboratory methods. <http://www.dcwater.com/waterquality/coliform.cfm>

### **Fecal coliform Indicators**

According to EPA:

Fecal coliforms, a subset of total coliform bacteria, are more fecal-specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper mill wastes.

This statement is extremely misleading. By 1898, Harvard medical School had documented that *Klebsiella* caused pneumonia, endocarditis, gangrene of the lung (necrotizing "flesh eating" infection), and acute otitis media.

The fecal coliform test is used to assure the safety of food, water and sewage sludge even though 95% of the non-heat resistant pathogenic members of the Enterobacteriaceae family will not show any growth at the elevated temperature of 112.1°F. While a positive coliform test with a positive fecal coliform test would be an acute violation for drinking water, and a positive fecal coliform test would cause the rejection of food, sludge / biosolids for use on grazing and agricultural land are allowed to contain two million colonies of heat resistant bacteria per gram of sludge / biosolids 24 hours after the laboratory test starts. Yes, the laboratory technician counts the colonies and reports them either as colony forming units (CFU) or most probable number (MPN) at the start of the test.

This is a deliberate attempt to fool the public as the pathogenic Enterobacteriaceae family as well as the other major pathogens multiply best at the human body temperature of 98.6°F or below. As long as food and water are readily available, with the appropriate temperature bacteria will continue to multiply. As noted earlier, the *E. coli* K 12 strain has been multiplying continuously for 89 years. *E. coli* and *Salmonella* in sludge has been documented to survive for 72 weeks on grazing land.

In 1904, Dutch physician and professor of physiology, Christiaan Eijkman, developed a fermentation test to isolate warm blooded animal and human fecal coli bacteria at 114.8°C as a means to quickly determine dangerous human fecal contamination of water. His theory was that fecal coli bacteria from cold blooded animals would not grow at the elevated temperature and had no sanitary significance.

In a February 2009, e-mail EPA's Richard Reding, Chief, Engineering and Analytical Support Branch, pointed out that the heat inactivation of most E. coli and reduction or eliminating other similar bacteria from the test was by design when he wrote that fecal coliform "are distinguished from the coliform group by their ability to grow at the elevated temperature of 112.1°F. Thus, "inactivation" is by design so as to eliminate those bacteria that are unable to grow at 112.1°F."

We now know Fecal coliform are only thermotolerant (heat resistant) Enterobacteriaceae coliforms, primarily E. coli, but may include Klebsiella, Citrobacter, Enterobacter, etc. We also know the elevated temperature of the test has no sanitary significance as all pathogens have optimum growth rates at normal body temperature or lower. There are two laboratory artificial culture matrices offered for enumerating E. coli which could lead one to believe a laboratory might have some choice in how well the bacteria grow in the test for sludge / biosolids.

EC Matrix Solution ATCC Catalog No.: 30-2501

EC Matrix Solution, With Reduced Growth Factors ATCC Catalog No.: 30-2503

No one wants to talk about the fact that artificial culture media only allows one percent of the viable bacteria to be detected. However, in the 2001 WHO document, "Water Quality: Guidelines, Standards and Health", the editors state:

The arbitrary definitions adopted for E. coli and the related coliforms were all based upon cultural characteristics, including the ability to produce gas from lactose fermentation (HMSO 1969). Hence, the thermotolerant coliforms include strains of the genera Klebsiella and Escherichia (Dufour 1977), as well as certain Enterobacter and Citrobacter strains able to grow under the conditions defined for thermotolerant coliforms (Figueras et al. 1994; Gleeson and Gray 1996). This phenotypic approach has also resulted in E. coli or a related coliform being ignored simply because they failed to ferment lactose, failed to produce gas from lactose or were indole-negative at 44.5°C. The approach had been repeatedly questioned (Waite 1997), and was only resolved in the UK in the 1990s (HMSO 1994). It has long been recognised that artificial culture media lead to only a very small fraction (0.01–1%) of the viable bacteria present being detected (Watkins and Xiangrong 1997).

[http://www.who.int/water\\_sanitation\\_health/dwq/iwachap13.pdf](http://www.who.int/water_sanitation_health/dwq/iwachap13.pdf)

WHO did not include the 1979 study, "Fecal coliform and E. coli estimates, tip of the iceberg", B. J. Dutka, et al.. In it Dutka found that E. coli, Klebsiella and Enterobacter enumeration testing results depended on the the age of the cultures and temperature. As an example testing at 95°, 106.7°, 109.4°, 112.1°, and 95°C for 4 hour followed by 18 hour at 112.1°F resulted in enumerating the least number of bacteria (5%) at the highest temperature to 100% at the lowest temperature. Water, Air, & Soil Pollution, Volume 11, Number 3, 349-362

There appear to be a lot of people who do not understand that the fecal coliform test can only indicate heat resistant members of the group as a whole. To clarify this, in 2007, I put the question to several sludge scientists at EPA and USDA as well as to EPA's microbiologist, Mark Meckes. Meckes was the only one who would admit he knew the answer. He stated, "most strains of Escherichia coli will ferment lactose under the elevated temperature test for fecal coliform and therefore will meet the definition of "fecal coliform." Similarly, some strains of Klebsiella will also ferment lactose under these same test conditions and will meet the definition of "fecal coliform"". Moreover, "Thermotolerant

strains/variants of virtually any of the Enterobacteriaceae would also be defined as "fecal coliform" as long as they produced acid and gas under the specified test conditions."

[http://thewatchers.us/FC\\_Meckes.html](http://thewatchers.us/FC_Meckes.html)

One hundred years after Eijkman proposed that only human thermotolerant (heat resistant) *E. coli* could show some biological activity at the elevated temperature of 114.8°F highly educated sludge researchers still appear to be confused about the nature of a fecal coliform bacteria. EPA's explanation of the fecal coliform test method 1681 reinforces the confusion by implying *E. coli* is something other than the primary heat resistant fecal coliform. EPA states:

"These methods use culture-specific media and elevated temperature to isolate and enumerate fecal coliform organisms. Fecal coliform bacteria, including *Escherichia coli* (*E. coli*), are commonly found in the feces of humans and other warm-blooded animals, and indicate the potential presence of other bacterial and viral pathogens."

However, EPA warns there could be problems with the matrix used to grow bacteria:

Based on the high false positive rates observed for Method 1681 in some matrices, EPA recommends that laboratories conduct their own matrix-specific comparisons to determine the most appropriate method (1680 or 1681).

It is not that EPA does not tell the truth in the test methods, you just have to read the complete document very carefully to find that *E. coli* colonies are counted, not single bacteria as reported in test results. which makes the test results inaccurate. Deep in the document EPA states:

"Fecal coliform bacteria are gram-negative, non-spore-forming rods that are found in the intestines and feces of humans and other warm-blooded animals. The predominant fecal coliform is *E. coli*. In this method, fecal coliforms are those bacteria that ferment lactose and produce gas within  $24 \pm 2$  hours in A-1 broth after incubation at  $112.1^\circ\text{C} \pm 0.2^\circ\text{C}$ . Since coliforms from other sources often cannot produce gas under these conditions, this criterion is used to define the fecal component of the coliform group."

The question that must be answered is what good is the test for fecal bacteria done at 112.1°F when the optimum growth temperature is 98.6°F? Not only that, but the attenuated (genetically modified) quality control strains will not grow at that elevated temperature. EPA requires that *E. coli* laboratory culture strain ATCC # 25922 (growth at 98.6°F – Biosafety Level: 1 ) be used as a positive control.

Laboratories are warned this strain is subject to genetic shift if it is transferred over 5 times.

*Enterobacter aerogenes* laboratory culture strain ATCC # 13048 (growth at 86°F – Biosafety Level: 1 ) is to be used as a negative control.

According to ATCC: Laboratories handling Biosafety Level 1 Cell Cultures should:

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. This cell line is not known to cause disease in healthy adult humans. These cells have NOT been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents, unless otherwise reported on the Certificate of Analysis. Regardless of results reported on the Certificate of Analysis Universal Precautions according to 29 CFR 1910.1030 should be followed at all times when manipulating these cell lines.

The term biological hazard or biohazard mean any viable infectious agent (etiologic

agent) that presents a risk, or a potential risk, to the well-being of humans.

BSL-1 risk group contains biological agents that pose low risk to personnel and the environment. These agents are highly unlikely to cause disease in healthy laboratory workers, animals or plants. The agents require Biosafety Level 1 containment. Examples of BSL-1 organisms are: *Agrobacterium radiobacter*, *Aspergillus niger*, *Bacillus thuringiensis*, *Escherichia coli* **strain K12**, *Lactobacillus acidophilus*, *Micrococcus leuteus*, *Neurospora crassa*, *Pseudomonas fluorescens*, *Serratia marcescens*.

Although highly unlikely to cause disease in healthy laboratory workers doesn't mean these biologicals won't when these organisms are spread in the environment (some as pesticides) and where they can pick up virulent genes. T.K McDaniel and J.B. Kaper documented in a 1997 study, the virulent 'pathogenicity island' from another bacteria can be picked up by *E. coli* K-12 in a single genetic step.

According to the Material Safety Data Sheet (MSDS), All *E. coli* are Hazard Group 2 bacteriological agents except *E. coli* O157:H7, which is Hazard Group 3. <http://www.ssi.dk/ProdukterYdelsler/SSI%20Diagnostica/Downloads/~media/Admin/Diagnostica%20Downloads/Downloads%20DK/Sikkerhedsdatablade/MSDS%20E%20coli%20strain1.ashx>

Yet, EPA and its partners claim it is safe for the public, especially children, to be exposed to Biosafety Level 2 and Level 3 pathogenic contaminated sewage sludge / biosolids / compost based on some mythical level of heat resistant fecal coliform, while telling laboratory personnel there is a risk in being exposed to small laboratory samples. EPA states in Method 1681:

The analyst must observe normal safety procedures required in a microbiology laboratory while preparing, using, and disposing of media, cultures, reagents, and materials, and while operating sterilization equipment.

Field and laboratory staff collecting and analyzing environmental samples are under some risk of exposure to pathogenic microorganisms. Staff should apply safety procedures used for pathogens to handle all samples.

Mouth-pipetting is prohibited.

EPA promotes the use of Biosafety level 2 and 3 pathogen contaminated sludge / biosolids and compost on parks, school grounds, home lawns and gardens where you and your children can be exposed without any warning, yet, even laboratory personnel are warned about the danger and they still get infected. Can you imagine a coliform outbreak in laboratories across 35 states at once? It happened. In April 2011, CDC made an investigation announcement: "Multistate Outbreak of Human *Salmonella* Typhimurium Infections Associated with Exposure to Clinical and Teaching Microbiology Laboratories". Clinical and teaching microbiology laboratories reported 73 individuals (mostly students or employees) infected with the commercially available attenuated outbreak strain of *Salmonella* Typhimurium in 35 states between August 20, 2010 to March 8, 2011. CDC states, "Sixty-three percent of patients are female. Fourteen percent of patients have been hospitalized. One death has been reported."

CDC recommendations:

- Be aware that bacteria used in microbiology laboratories can make you or others who live in

your household sick, especially young children, even if they have never visited the laboratory. It is possible for bacteria to be brought into the home through contaminated lab coats, pens, notebooks and other items that are used in the microbiology laboratory.

- Persons working with infectious agents, including Salmonella bacteria, must be aware of potential hazards, and must be trained and proficient in biosafety practices and techniques required for handling such agents safely,

- All students and employees using the laboratory should be trained in biosafety practices.

<http://www.cdc.gov/salmonella/typhimurium-laboratory/042711/>

### **The last two indicators are Gram-positive cocci.**

Like E. coli, coliform and fecal coliform, only low levels of chemical disinfectants, heat or cold are required to inactivate the Streptococci and Enterococci indicators. Inactivate does not mean kill or destroy. Biological samples can be purchased freeze dried.

### **Fecal Streptococci Indicator**

According to EPA:

Fecal streptococci generally occur in the digestive systems of humans and other warm-blooded animals.

The term Fecal streptococci (Streptococcus) indicator is misleading because it implies it is not a pathogen. Nothing could be further from the truth. Streptococcus is the cause of acute glomerulonephritis (a type of kidney disease), strep throat, meningitis, bacterial pneumonia, dental abscesses, endocarditis, erysipelas, impetigo, cellulitis, hepatic necrosis in horses, mastitis in cows, invasive GAS disease, otitis media, reactive arthritis, rheumatic fever, septicemia, streptococcal toxic shock syndrome, scarlet fever, sexually transmitted urogenital infections, strangles in horses, systemic bacteremia, tooth decay, death and it was the first bacteria associated with necrotizing fasciitis (so-called 'flesh-eating' bacterial infections).

### **Fecal Enterococci Indicator**

According to EPA:

Enterococci are a subgroup within the fecal streptococcus group. Enterococci are distinguished by their ability to survive in salt water, and in this respect they more closely mimic many pathogens than do the other indicators.

Formally, Type D Streptococcus. Enterococci is an example of a non-pathogenic probiotic turning into a deadly pathogen. In the past, some enterococcal strains have been used as human probiotics. However, the first vancomycin-resistant enterococcus (VRE) was found in 1986. Now it is a major cause of antibiotic resistant health-care infections. The Enterococci is a cause of urinary tract infections, bacteremia, diverticulitis, meningitis, septicemia, endocarditis, wound infections, appendicitis and death. It also causes spoilage of heat-treated, packaged processed meats.

### **Conclusion**

EPA, FDA, USDA and many states have put public health and the economy at risk by promoting one

hundred year old science in their regulations for food and water as well as for sewage sludge / biosolids used as fertilizer for food crops and recycled sewage water used for irrigation of food crops. Studies prove these Agencies have known for well over 30 years that sewage treatment processes don't kill pathogenic bacteria and that antibiotic resistant bacteria are released in treated sewage water and sludge.

As the foodborne illness outbreak numbers have dramatically increased since 1986, it is a clear indication that the coliform and fecal coliform tests have failed to protect public health from these deadly bacteria in food and water. While the victims may spread disease and die in the hospital, deaths from community acquired infections caused by Enterobacteriaceae, pneumonia, bloodstream infections, and urinary tract infections are not counted as a part of the 1.7 million health care acquired infections that resulted in 98,987 deaths.

The Agencies have deliberately misled the public about the nature of the pathogenic members of the Enterobacteriaceae family of disease causing organisms which cause 40% of 1.7 million health care acquired infections. These are the Enterobacteriaceae animal, plant, soil and water members known to infect humans: *E. coli*; *Citrobacter*; *Enterobacter*; *Klebsiella*; *Shigella*; *Salmonella*; and *Yersinia* as well as the less well known families of *Averyella*; *Budvicia aquatica*; *Buttiauxella noackiae*; *Calymmatobacterium*; *Cedecea*; *Edwardsiella*; *Ewingella*; *Hafnia alvei*; *Kluyvera*; *Koserella*; *Leclercia adecarboxylata*; *Leminorella*; *Moellerella wisconsensis*; *Morganella*; *Pantoea*; *Photobacterium*; *Proteus*; *Providencia*; *Rahnella aquatilis*; *Serratia*; *Tatumella*; *Xenorhabdus*; and *Yokenella regensburgei*. by claiming they were non-pathogenic coliform when tested at 95°F or non-pathogenic fecal coliform when tested at 112.1°F.

The tests for coliform and fecal coliform were designed to suppress those disease causing organisms in food, water or sewage it doesn't want to find. As the studies show, the higher the temperature, the less biological activity is shown by the fecal indicators. The tests for fecal contamination indicators such as coliforms, fecal coliforms, *E. coli*, *Streptococci*, *Enterococci* are inexpensive, but then fail to offer any protection when used outside public drinking water systems, especially when used for testing treated "fecal sewage products" such as sludge / biosolids fertilizer and recycled sewage irrigation water used on grazing land, food crop production land, parks, school grounds, home lawns and gardens.

Studies show these fecal contamination indicators only require a low level of disinfection to temporarily inactivate biological activity without actually killing them. This gives a false sense of security as many disease causing organisms require a high level of disinfection. Not only that, but bacteria recovering from insufficient disinfection become resistant to the disinfectant.

With so many strains of pathogenic *E. coli* causing disease, long term health effects and deaths, claiming most strains will not generally cause disease is not just a myth, it is a lie. It is self-evident that any reputable regulator, scientist or engineer should be aware that coliforms are the pathogenic Enterobacteriaceae family growing in medical tests at 98.6°F – which is the temperature of the gut. Furthermore, they must know that the fecal coliform test at 112.1°F is useless considering the fact that with minor exceptions, 99% of Enterobacteriaceae are inactivated.

EPA documents as well as other Agency documents and scientific studies show food, water and sludge experts are knowingly perpetuating the myths concerning *E. coli*, coliforms and fecal coliforms at the

expense of public health and the national economy. While we may not be able to completely protect ourselves from all disease causing organisms, we can prevent many of them by keeping contaminated sewage products (sludge / biosolids / reclaimed water) away from animals, off food crops and out of the drinking water. It is time we call on Congress to bring these agencies into the 21<sup>st</sup> century.

