Chapter ? Open for review

Are Scientists Unaware That Escherichia Coli Is A Pathogenic Coliform And Also A Fecal Coliform?
Tests for Enteric Coli-like-forms of Bacteria in Sludge, Food, and Water
A Review

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Help for Sewage Victims

In the current Environmental Protection Agency (EPA) and Center for Disease Control (CDC) literature the Agencies claim that even microbiologist are confused by the facts that some Escherichia (E.) coli are pathogens and some are laboratory cultured nonpathogens used as indicators for water contamination. E. coli K-12 is an indicator which has been cultivated in the laboratory so long it has become nonpathogenic. It has emerged as the work horse of the genetic engineering field after scientists discovered a method in the 1970s to induce antibiotic resistant genes into bacteria as a marker. The marker is used to confirm the transformation of the target bacteria with new DNA. This made it possible to create bacteria never before seen in nature such as E. coli 0157:H7.

After reviewing over 100 years of medical studies, and specifically those concerning the testing procedure, it is difficult to accept the conclusion that a microbiologist with a Ph.D is still confused about the pathogenic nature of E. coli. Yet, in reviewing current sewage and water studies, it does appear the current sewage and water scientists as well as their testing laboratories are unaware that E. coli is a disease causing organism as well as the primary member of the coliform group and the extremely small thermotolerant fecal coliform group. Could it be because these sewage and water scientists are only allowed to work with laboratory cultured nonpathogenic indicator coli-like-forms of the common clinical enteric members of Enterobacteriaceae? Clinical pathogenic members include, but are not limited too: Citrobacter, Edwardsiella, Enterobacter, Escherichia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Serratia, Shigella, Yersinia--includes Black Plague. However, that does not explain why they would not know studies show the thermotolerant fecal coliform test enumerates less than 5% of the fecal E. coli/Klebsiella bacteria. These are pathogens, not indicators, since they completely ignore 95% of the E. coli/Klebsiella as well as many pathogenic fecal bacteria and other disease organisms in sewage sludge and recycled/reclaimed water, drinking water and food.

One hundred and fifty six years after the great London Vibrio cholera outbreak, sewage and water scientists claim cholera and other disease causing coliform bacteria growing in shit (feces or fecal material) at normal body temperature 37°C (98.6°F) are not of fecal origin in water. It is their contention that actual fecal coliform contamination must be confirmed by growth under a controlled temperature of 44.5°C (112.1°F), which suppresses all nonthermotolerant bacteria. The final confirmation step is to verify the presence of E. coli. The claim is that if no E. coli is present there are no fecal pathogens present in food or water. A major problem with that is, according to CDC, there are 3,520 unique strains of E. coli O157:H7 that don't normally show up in the fecal coliform test.

Dr. John Snow recognized in 1854 that exposure to human fecal material in drinking water was very dangerous and deadly as the resulting London Vibrio cholera outbreak demonstrated.
E. coli was identified as the first known fecal pathogen to cause the deaths of newborns and their mothers in 1985. Klebsiella was the only other bacteria to have been described associate with illness and in feces. Originally they were referred to as Colon or coli bacillus or the Bacillus (B) coli cummunis group and Bacillus (B) aerogenes capsulatus. As the science matured, colibacillus became E. coli and the group expand to include many gram negative coli-like-forms that fermented lactose to produce gas and/or acid within 24-48 hours. These became the tribe Eschericheae and finally the pathogenic family Enterobacteriaceae. There is an exception, veterinarians still call use the term colibacillus for an E. coli infection that kills the very young.

They were all classified as coli-like-forms when incubated at the optimum growth temperature of 37°C (98.6°F). They were simply disease causing coliforms in the medical field. Coliforms were adopted by the Public Health Service in 1914 in a specific test to evaluate potential fecal contamination of food and water. The thermotolerant fecal coliform test for E. coli and Klebisella at 44.5°C (112.1°F) was developed in the belief that the high temperature suppressed bacterial growth from cold blooded animals, plants, soil and water that caused a false positive in the coliform test.

However, in the last 100 years, many animal, plant, soil and water bacteria have picked up pathogenic and antibiotic resistant virulent genes. As a result, at least 30 species of the coliform group are now enteric pathogens. Some pathogens also show up as false positives in the coliform test. Both coliforms and noncoliforms are suppressed by the fecal coliform test as studies show enteric pathogen growth, including E.coli and Klebsiella, is severely inhibited above 40°C and stops at about 45°C. While the bacteria are suppressed in the test, they are still viable, but they can not be cultured by standard method. The most important point is that scientists do know that the bacteria in sludge, water and food are still growing and multiplying at normal temperatures. Moreover, studies done during the last 100 years are convincing evidence there is no reason for regulators or scientists to be confused.

Background

We have been investigating sludge use on agricultural land since 1989 when Kansas City, Missouri created its sludge farm adjacent to the Alice Minter Trust farm. The City generously offered to furnish, and apply, sludge as a fertilizer. However, it could not furnish a comprehensive list of chemicals and organisms in the sludge. Nor could it explain why dairies across the country who accepted sludge, or were adjacent to sludge farms, were losing excessive numbers of cows. We knew disease causing organisms on farms was a problem particularly mastitis (women and animals), colibacillosis and Johne's disease, (e.g. chronic wasting disease) caused by Mycobacterium paratuberculosis. Studies and the laws did indicate sludge use was not safe as E. coli and Salmonella survived on grazing land for over 72 weeks. Moreover, the Solid Waste Act states that any infectious organisms in sewage sludge would cause it to be classified as a hazardous waste.

When we wrote the first peer reviewed paper "Sludge Disposal: Sanitary Landfill-Open Dump-Superfund Site?" against toxic sludge use on agricultural land in 1992, it was based on the EPA's 1989 proposed 503 sludge rule which listed 25 families of infectious pathogens in sludge, only five

were bacteria, Campylobacter, Escherichia, Salmonella, Shigella, and Vibrio Cholerae. While three of the five bacteria, E. coli, Salmonella and Shigella are classified as coliforms, only thermotolerant E. coli is considered to be a fecal coliform.

Today, EPA and CDC imply E. coli K-12 is the only strain found in water tests and it is not a pathogen. However, even if that was the case, as T.K McDaniel and J.B. Kaper documented in 1997 study, the virulent 'pathogenicity island' from another bacteria can be picked up by E. coli K-12 in a single genetic step. Moreover, it would not be logical for EPA tests to look for a nonpathogenic E. coli marker in water when there are so many infectious strains. According to CDC, "Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including E. coli O157:H7" are laboratory Risk Group 2 infectious agents. Even though infections may lead to death, CDC states they are "rarely serious and treatment is often available." There is no antibiotic treatment recommended for E. coli 0157:H7 as there is an increased risk of hemolytic-uremic syndrome (HUS) and death from its use.

What we didn't know at the time was the high temperature fecal coliform test stresses bacteria which results in the suppressed growth of E. coli and stops the growth of coliform pathogens such as Salmonella and Shigella. While the high temperature stops the bacterial growth due to stress, the bacteria remain viable but they are nonculturalable by standard methods. The test ignores Campylobacter, E. coli 0157:H7 and Vibrio as well as many other pathogens such as the old Superbugs Enterococcus faecium, Staphylococcus aureus (MRSA), Acinetobacter baumannii, and Pseudomonas aeruginosa, Moreover, it actually suppresses or ignores many more pathogens including the Superbugs Klebsiella species and Enterobacter species. There are now new Superbugs spreading around the world such as Clostridium difficile (C. diff), ESBL-producing E. coli, New Delhi metallo-beta-lactamase, or NDM-1 producing genes in Escherichia coli and Klebsiella pneumoniae and Aspergillus fungus. The newest killer strain is E. coli ST131 found mostly in urinary tract infections.

The list of pathogens was removed from the final Part 503 sludge rule in 1993, and EPA's website, because the inherent infectious characteristics required sludge to be treated as a hazardous waste under the Solid Waste Act. EPA has reserved chapters in the Hazard Waste regulation Part 261 for the treatment of infectious waste (Appendix V) and disease causing organisms, e.g. etiologic agents, (appendix VI). Due to the etiological agents in sludge it is illegal to transport it in interstate shipments under Part 72.3 unless properly labeled and packaged -- unless it is classified as a biological product. This appears to be the real reason the industry adopted the term biological solids for sludge (e.g. biosolids).

EPA does state in 503.9 of the sludge rule that exposure to the pathogenic organism pollutants in sludge through direct contact or indirect contact through the air, food and water could cause death, disease, cancer, etc.. Scientists know the fecal coliform test gives no indication of the number or type of growing pathogens in bulk or packaged sludge at normal temperature. They know the high heat of the fecal coliform test suppresses, or ignores, all pathogens except thermotolerant forms of E. coli and Klebsiella. They also know EPA allows the laboratory technician to count up to two (2) million thermotolerant colonies per gram (Class B) and up to one (1) thousand thermotolerant colonies per gram (Class A) and report each multigeneration colony that can be seen with the naked eye as one individual bacteria (most probable number (MPN)) or colony forming unit (CFU). Yet, many scientists continue to promote sludge and publish studies

stating all pathogens are destroyed and the regulations are strictly enforced to protect public health.

Bacillus globigii. Emu tracer is comparable size to coliform bacteria. Colonies after incubation for 24 hours can be counted with the naked eye. Sample are diluted by multiples of 10 until they may be counted. Courtesy of http://www.environmentaltracing.info/BacteriaTracers.html

We found that was not true in 1998. The Alice Minter Trust farm had received runoff from Kansas City, Missouri's sludge farm for eight years. We suspected it had become contaminated. After meeting with James Macy, Missouri DNR, and John Dunn, Region 7 Sludge Coordinator, Macy agreed to test for metals and Dunn agreed to test for pathogens. On the appointed day, Dunn refused to participate. When EPA refused to do the bacterial soil test we could only find one EPA certified laboratory in Pittsburg, Kansas that would agree to run the standard soil tests, Quality Water Analysis Laboratory, Inc. (QWAL). In a sample taken from the easement across the sludge site leading into the farm fecal coliform was found at over 650,000 colonies per 100 grams of soil. On the actual farm one soil sample revealed E. coli at over 800,000 colonies per 100 grams of soil after growing on the test media for 24 hours at an incubation temperature of 37C (98.6F) , while indicator fecal coliform levels were very low at only 3,000 colonies per 100 grams of soil after growing on test media for 24 hours at 44.5C (112.1F).

In a separate test sample taken from the opposite end of the field, Salmonella was also found at over 800,000 colonies per 100 grams of soil while fecal coliforms were 9,000 colonies per 100 grams of soil. At the time we did not understand that fecal coliforms were heat inhibited E. coli and other coli-like-forms which only indicated fecal pollution from a field of sewage fecal material. However, we did understand growing food crops on soil that was contaminated made the Alice Minter Trust and the buyers financially responsible for any damages to health caused by the products. Yet, it was Macy's opinion that we should go ahead and farm the contaminated land because EPA had no standards for pathogens in soil. Therefore, we decided it was our duty to our children and grandchildren find out what EPA and the industry knew and when they knew it.

In 1996 we wrote a review for the National Sludge Alliance of the 1996 National Academies of Sciences' National Research Council (NRC) Committee's report "Use of Reclaimed Water and Sludge in Food Crop Production". Immediately after part 503 was released, EPA and others funded the report to support the science behind the sludge rule risk assessment. As stated in the Preface "At the time [1993], EPA was just finalizing the Part 503 Sludge Rule, and one of the major implementation concerns was with the food processing industry's reluctance to accept the practice." There was a direct conflict of interest as the chair, Al Page, also co-chaired the part 503 peer review committee approving the science. It was apparent the Committee only reviewed information supplied by EPA to support the part 503 sludge rule. The finding on health conflicted with our research and EPA studies as well as documents, many of which we did not find at that time. The Committee's conclusion was less than approving. It wrote, "The suite of existing federal regulations, available avenues for additional state and local regulatory actions, and private sector forces appear adequate to allow, with time and education, the development of safe beneficial reuse

of reclaimed wastewater and sludge."

This was followed in 1998 by a limited edition review of our research on sludge use, "DEADLY DECEIT: Our Children at Risk From Sewage Sludged/Biosolids." It was evident at that time that there was no science behind the sludge rule and absolutely no concern for public health. We noted in Chapter Nine, Risky Risk Assessment, that in a 1997 working paper appraising the part 503 sludge rule risk assessment Ellen Z. Harrison, Murray B. McBride, and David. R. Bouldin of Cornell Waste Management Institute stated, "-- there are fundamental errors in the assessment structure, a number of untenable assumptions made, and serious omissions (whether due to oversights or data gaps) which result in regulations that are not sufficiently protective." In response, Bob Persiasepe, the EPA Assistant Administrator for Water, was concerned about "the potential negative impact on the many benefits that New York citizens can realize from the beneficial use of biosolids." Furthermore, he said, "It could unnecessarily alarm citizens about the threats to public health and the environment that the draft claims may occur from the use of biosolids."

We Were Not Alone

Government Agencies and sewage scientists claim they don't have a clear understanding of the health and environmental damage caused by spreading disease causing organisms in the environment. These disease causing organisms are spread through drinking water, sewage effluent released to surface water, sewage overflows or recycled for food crop irrigation and sludge (biosolids) spread on agricultural grazing and crop land. Drinking water treatment plants are allowed to fail 5% of the required monthly test for coliforms. Plus, it would be another 24-48 hours before it test results for E. coli are known. Recycled sewage effluent used for food drop irrigation is also allowed to contain a certain amount of coliforms. Sewage sludge (biosolids) is allowed to contain 2 million thermotolerant colonies per gram counted at the end of the 24 hour fecal coliform test. Not only does the test suppress most coliforms and ignore other disease causing organisms, the thermotolerant colonies of bacteria are actually reported as individual bacteria.

According to CDC, in 1995-1996, an estimated 6.5-33 million persons became ill from foodborne diseases, and up to 9,000 died. CDC raised the estimate to 76 million foodborne illnesses in 1999 and dropped the death rate to 5,000. Then it stopped counting. Yet, Ralph J. Touch, Chief Sanitarian for the federal Health and Human Services, sounded the alarm on foodborne illnesses in a 1996 presentation in Scotland. He warned there were 80 million cases annually. His warning was ignored by U.S. scientists even though in the EPA's 1986 landfill risk assessment for pathogens, microbiologist Charles Gerba estimated there were only 1-2 million foodborne illnesses a year caused by Salmonella. He noted Salmonella survived on grass treated with sludge in Switzerland for 16 months He also acknowledged that coliforms, fecal coliforms and fecal streptococci could cause disease. The question no sewage scientist wants to answer is, why the sudden explosion in foodborne illnesses over a ten year period?

There were scientists and others looking for answers. Help for Sewage Victims was formed in the early 1990s to gather and disseminate information on sludge and victims its by Linda Zander after their dairy herd started having health problems. The National Sludge Alliance was created in 1996 by local groups across the United States with the help of EPA's Hugh Kaufman, Abby Rockefellow, and Laura Orlando. Charlotte Hartman was chosen to be the National Coordinator. The Alliance

called on Congress to halt sludge dumping as a fertilizer. A number of fact sheets exposing the corruption and danger of sludge use were issued over several years. Later, individuals of local organizations started up websites such as Caroline Snyder's http://sludgefacts.org which collects studies, relevant court cases and news stories. Snyder also wrote the 2005 peer reviewed history paper "The Dirty Work of Promoting “Recycling” of America’s Sewage Sludge." Helane Shields website http://sludgevictims.com focuses on collecting data on sludge victims across the country.

Cornell scientists were the first to draw the wrath of EPA down on them when Ellen Harrison, Murray B. McBride and David R. Bouldin at Cornell's Waste Management Institute wrote the "Case for Caution" in 1997/1999 focusing on the high levels of both toxic metals and pathogens in sewage sludge. They suggested the sludge rule should be more restrictive to match those of other countries and EPA should have taken a "do no harm" approach in its risk assessment. USDA reviewed the paper for EPA and commented farmers were already using toxic materials and contaminated manure, in effect, that agricultural use of manure was more dangerous. The result was EPA tried to force Cornell administrators to withdraw the "Case for Caution" and fire the authors. In spite of the potential lost of federal funding that go to Land Grant Colleges and Universities, Cornell administrators refused to cave in to EPA pressure. Harrison was the lead author for the 2002 article "Investigation of Alleged Health Incidents Associated With Land Application Of Sewage Sludges." Harrison also served on the 2002 National Academies of Science (NAS) "Committee on Toxicants and Pathogens in Biosolids Applied to Land, National Research Council" which wrote "Biosolids Applied to Land Advancing Standards and Practices". According Harrison, the Committee drew heavily on David Lewis' unpublished manuscripts which identifying various areas of concern needing additional research, then NAS removed references crediting his work without consulting the panel. Cornell Waste Management Institute continues to research sludge with a statement of caution "There are risks and benefits associated with the nutrients, organic matter, chemical contaminants and pathogens they contain."

EPA microbiologist David Lewis was targeted by sludge regulators and sewage scientists after a commentary published in Nature in 1996, titled “EPA Science: Casualty of Election Politics.” Some of EPA's "irrational approach" to protecting public health when he wrote "Sludge Magic at EPA" for The Journal of Commerce in 1999 in which he mentions the part of the results from the Alice Minter Trust soil tests. He also noted the Agency's doublespeak on pesticides, the lack of interest in microorganism could lie dormant in soil for years, the lack of interest in antibiotic resistant bacteria and the lack of Congressional and media interest in how the ocean dumping problem was solved. Lewis' seven year campaign to change EPA sludge policy from within resulted in a "whitepaper" to blacken his name by EPA sludge expert John Walker and industry associates. It is interesting to note that while Walker first documented lime treatment of sludge only caused Salmonella to go dormant for about 30 days, now that he is a regulatory sludge promoter, he becomes intent on destroying Lewis's career for revealing some of the same information he did in 1973 and eventually forcing him out of the EPA. Before Lewis' forced retirement, he investigated children's deaths associated with pathogen contaminated sludge sites as well two dairy farms destroyed in Georgia where sewage scientists faked a study. He was also involved in two studies detrimental to sewage scientists at EPA and in the sludge industry: "A High-Level Disinfection Standard for Land-Applied Sewage Sludges (Biosolids)" and "Interactions of pathogens and irritant chemicals in land-applied sewage sludges (biosolids)". Lewis' real problem was the discovery that accepted treatment processes don't work on some pathogens, specifically the survival and transfer of HIV virus on sanitized dental instruments in 1992 as outlined in Lancet article, "Cross-contamination potential

with dental equipment."

Lewis's most striking discovery was using ultrasound to break up sludge biofilms (biosolids) thereby releasing all the pathogens thought to be killed during the treatment process. Fine particles are created during treatment as bacteria break down the organic matter in sewage. Biofilms are the result of stressed bacteria releasing slime polysaccharide, which bind bacteria, viruses, parasites, protozoa together with the remaining fine inorganic and organic particles. In effect, biological solids or biosolids = "slime bulking" or sludge. This directly conflicts with the Agency and Industry claim that chemicals and toxic metals were permanently bound by the slime. Lewis reported some of his findings during a 2004 Hearing by the Committee on Resources, Subcommittee on Energy and Minerals U.S. House of Representatives titled "The Impact of Science on Public Policy" where he discussed the corruption of science at EPA.

Lewis wasn't the first to discover that sewage treatment did not kill pathogens. In a 1991 German study by D. Strauch, Institute of Animal Medicine and Hygiene, University of Hohenhiem, "Survival of pathogenic micro-organisms and parasite in extreta, manure and sewage sludge", he said, "sewage sludge is rightly described as a concentration of pathogens" because "most pathogenic agents can survive the treatment process" and the sewage treatment process causes some of the pathogenic disease organisms to be absorbed or enclosed in faecal particles. Moreover, it was reported that two groups of researchers had found pathogenic disease organisms will be taken up inside food crops. Furthermore, "In any case, the agricultural utilization of hygienically dubious sewage sludge poses a risk for the whole national economy."

Nadya Markova and associates at the Institute of Microbiology, Bulgarian Academy of Sciences, found in a 2010 study that even starved weaken vegetable cell E. coli K-12 could survive one hour in boiling water at 100°C and 15 minutes of autoclaving at 134°C. Recovery to normal growth was accomplished in 2 weeks by incubating at 37°C. This would tend to prove EPA has no treatment process for sewage effluent and sludge that will kill all disease causing organisms or protect public health.

By 1953, it was known mesophilic sporeforming strains could be transformed in thermophilic sporeforming bacteria. In her review, Mary Belle Allen, Hopkins Marine Station of Stanford University, noted that scientist suggested that bacteria which grew between 40 and 45C should be designated "thermotolerant" forms. She found thermophilic sporeforming bacteria generally could show some activity between 35°C and an upper range of 60-70°C. Of considerable interest is her revelation that these thermophilic sporeformers caused spontaneous combustion fires in hay piles. We now know they also cause spontaneous combustion fires in stored sludge piles.

We should at least mention the noncoliform pathogenic Bacillus and Clostridium strains are among the sporeforming genera that als survive well when stressed by lack of moisture, food, radiation and heat. They can survive at well over 100°C. Not only that but they survive radiation treatment. As spores in sludge (or biosolids), the bacteria can survive in soil for years, or even centuries if necessary, until the proper moisture, food and temperature are available.

One hundred years ago, Daniel D. Jackson, laboratory Division, Department of Water Supply, Gas and Electricity, New York City, noted in the Journal of the American Journal of Public Health (that

much like today), some scientists refuse to acknowledge to the public that coliforms (e.g., coli-like-forms) are of sanitary significance. He stated:

> "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."

E. coli (Coli Bacillus or B. coli) was identified in 1885 by the German pediatrician, Theodor Escherich. According to the Federal Drug Administration (FDA), F. Shardinger proposed that B. coli should be used as an indicator of fecal pollution of water in 1892. Christiaan Eijkman suggested in 1906 that the only E. coli of sanitary significance in feces from warm blooded animals would grow at 46°C (114.8°F). It is difficult to understand the scientific concept in that farm animals normal temperature is around 101.3°F to 103°F, while a human's is 98.6°F. Scientists later determined that E. coli found at the lower temperature of the human body would be from cold blooded animals with no sanitary significance. Shardinger, Escherich and Eijkman thought exposure to human feces in water was very dangerous due to the early identification of cholera being transmitted through drinking water.

It is impossible to believe there is still scientific confusion over the coliform or fecal coliform test after 104 years and hundreds of millions of dollars spent on studying these bacteria. As noted above, scientists have been questioning this opinion for 100 years and the tests themselves for the past 80 years. Even the National Academies of Science reviews (claimed studies) have questioned the validity of EPA's testing procedures -- but failed to elaborate on the lack of science. We know the optimum temperature for growth of pathogenic bacteria is the human body temperature of 98.6F. Unlike bacteria which become stressed and go dormant at high temperatures, when the human core body temperature rises about 107°F, heat stroke or death will occur. Yet, FDA still uses 45°C for testing shellfish and 44.5°C for other foods. EPA and CDC documents attempt to create confusion in the public mind with their description of E. coli, even when they knew that no natural forms of E. coli are harmless outside the human gut. Both EPA and CDC use the following description for E. coli:

> "Escherichia coli (abbreviated as E. coli) are a large and diverse group of bacteria. Although most strains of E. coli are harmless, others can make you sick. Some kinds of E. coli can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses. Still other kinds of E. coli are used as ["]markers["] for water contamination—so you might hear about E. coli being found in drinking water, which are not themselves harmful, but indicate the water is contaminated. It does get a bit confusing—even to microbiologists."

The key word in this description is "markers" or indicators (e.g., nonpathogenic surrogate strains)

such as Bacillus globigii spores and Fluorescent microspheres for coliform which do not yet exist naturally in the environment. They are also called "tracers". Markers have one thing in common. While they are nonpathogenic, they carry antibiotic resistant genes as a means to separate them from natural bacteria which will be suppressed by antibiotics. On the other hand antibiotic resists genes are used by genetic engineers as "markers" to establish that the transfer of desired genes has been accomplished. On that, there is no confusion to microbiologists. Moreover, EPA tests do not differentiate between E. coli enteropathogenic, enterotoxigenic, enteroinvasive strains, genetic strains engineered for commercial applications and those cloned nonpathogenic cultured laboratory strains used as drug deliver systems as well as tracers (markers) that are derived from E. coli K12. L. W. Sinton described two genetically modified antibiotic resistant clones in 1980 used to trace sewage pollution in groundwater. They were the lactose negative E. coli J6-2 which would not show up as a coliform and the lactose positive E. coli PB 622.

Sinton also had a warning for us. Nonpathogenic bacteria tracers with antibiotic resistants genes are not necessarily benign. Antibiotic resistance genes (R-factor) was wide spread among coliform and fecal coliform. Moreover, "E. coli was the most frequent cause of infections among hospital patients." If they are ingested they may transfer resistance to normal gut flora or any antibiotic sensitive bacteria a person may become infected with. It has since been documented these antibiotic resistant bacteria in the gut may remain so for over four years. It has also been documented that as these bacteria move through sewage treatment plants a higher percentage leave the treatment process than entered it.

For their own safety researchers are not allowed to use pathogenic organisms as markers which are deliberately released in water or the environment as a part of their research on contamination. Most marker cultured strains also survive poorly in the environment. Researchers are well aware that deliberately releasing pathogenic organisms into water or the environment could be classified as an act of agricultural bioterrorism.

Contending that most strains of E.coli are harmless is like saying most bullets are harmless. E. coli was the first opportunistic pathogen identified. What that means is, if that harmless E. coli gets into any part of the body outside the gut such as the blood, brain or heart, it can mess up the rest of your life. Other types of E. coli just do it a lot quicker. Between the animal, human, plant, soil and water bacteria swapping virulent genes in the gut as well as in sewage and drinking water treatment plants, a high temperature fecal coliform test to indicate human fecal contamination does nothing to protect food, water or pubic health.

We depend on EPA and our laboratories to keep up with current science, especially on pathogens and the diseases they cause. But, according to the Environmental Health Division, Wisconsin State Laboratory of Hygiene, Water Microbiology for State and Federal Agencies,

> "Total Coliforms are indicator organisms used to detect bacterial contamination in drinking water. Their presence indicates that a pathway for contamination exists and organisms that cause disease may be present, even though total coliforms themselves typically do not cause disease in healthy individuals."

Yet, there are now at least 29 coliforms, besides E. coli, that will cause disease in healthy individuals.

A second part of the myth by EPA is this definition:

> "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."

Yet, Klebsiella has become one of the enteric Superbugs.

University of California scientists have now exposed the fraudulent tests required by EPA. They state:

> "Whether talking about Good Agricultural Practices or TMDL's (Total Maximum Daily Loads) in ag-runoff water, developing fruit and vegetable microbial standards, food safety management and certification plans, or setting regional water policy, basing decisions on total numbers of 'Coliform' bacteria or 'Fecal Coliforms' is not supported by current science. These days, there is a lot of talking and a lot of confusion. It may be helpful to look at Figure 1 and realize that all 'Fecal Coliforms' are also 'Coliforms' and some Fecal Coliforms are non-pathogenic E. coli and some are pathogenic and toxigenic E. coli . Some pathogens, such as Salmonella are 'Coliforms' but don't give a positive result in tests for 'Fecal Coliforms'."

130 Years of Science Associated with the Coliform and Fecal Coliform Test

Dr. John Snow was the first to use common sense to assume that a polluted public well in London was a source of the Cholera epidemic of 1854. His theory that drawing sewage polluted water from the Thames down river from London was the source of the Cholera outbreak. However, further research indicated that an infant girl's diarrhea was dumped in a leaking cesspool three feet from that public well. It was later established that the outbreak was over by the time the pump handle was actually removed. However, removing the pump handle stopped a second outbreak when the father of the infant came down with the Cholera the same day the pump handle was removed. Both the infant and the father died. Unfortunately, the source of the infants infection was never determined. While he did not know the Vibrio bacteria was the cause of the Cholera outbreak, he appeared to prove his point by mapping the people exposed and removing the handle of the public well serving the area. Snow was able to make this common sense deduction because the part of London not affected by the outbreak was served by a second water company which drew its water from the Thames above the city.

In the early scientific literature enteric coli-like bacteria were identified as either gram-negative Bacillus (B.) coli or the encapsulated Bacillus (B.) aerogenes (gas producing). Over the last century E. coli replaced B. coli as the prime fecal bacteria and it and other coli-like bacteria became members of the family Enterobacteriaceae in the scientific literature. It appears that the encapsulated gram negative B. aerogenes became, Aerobacter aerogenes, Enterobacter aerogenes, Pasteurella aerogenes and Klebsiella aerogenes. Science has shown this group of bacteria may have variants that transform from aerogenic to anaerogenic (gas producing -- non-gas producing) depending on the culture time as well as the opposite transformation. Salmonella

also falls into that category.

No one seems to know the first name of von Fritsch who is said to have described Klebsiella pneumonia and K. rhinoscleromatis from feces in 1880. It was isolated from a pneumonia patient in 1982 by Carl Friedländer, German pathologist, and became known as Friedländer's or Friedlinder's bacillus as well as Bacillus aerogenes. Theodor Escherich discovered B. coli in 1885 which was later named after him in 1919.

By 1903, WG Savage, MD, was aware E. coli was not necessarily an indication of fecal pollution. He recognized the varying virulence of human B. coli and assumed that E. coli from other sources was not virulent in water. The following year, Christiaan Eijkman,Dutch physician and professor of physiology, proposed his fermentation test at 46°C as a positive means of separating fecal pollution from humans and other warm blooded animals from cold blood animals and other sources.

In 1907, Eugene F. McCampbell, Instructor in Bacteriology, Ohio State University, Columbus, illustrated the potential for transmitting disease in a report on bacteria in public drinking cups. In the report, The Public Drinking Cup, 59 cultured bacteria were taken from public drinking cups at ten different public water sources. Some bacteria were unculturable, but 26 species of bacteria were found. While the identification has changed over the last 100 years, some appear to be pus causing Staph bacteria and Strep bacteria. The virulence of Staphylococcus pyogenes albus was tested by infecting guinea pigs. The virulence was not judged to be a great risk as it took 7 or 8 days to kill the pigs. The virulence of Bacillus sporogenes was much greater, it killed the guinea pigs in 48 hours. This appears to be Clostridium sporogenes. Bacterium tuberculosis had been documented in cups as had the transmission of syphilis. One hundred years later we know the cause of syphilis, Treponema pallidum, is still difficult to culture, if at all. Bacillus coli was found at only two of the 10 drinking water sources. McCampbell acknowledged that Bacillus coli could be found in healthy mouths, even though it was normally an intestinal bacteria. He did note Bacillus coli was found in a well along side a well traveled country road. McCampbell didn't think this was an indicator of fecal pollution, because the nearest outhouse (toilet) was 30 feet downhill from the well. He gave no indication of how deep the well was dug.

Daniel D. Jackson reported in 1911 that there were seventeen known varieties of B. coli. Thirteen had been isolated from feces or diseased conditions. Seven varieties were isolated from water. His main points were that there was no "typical" B. coli as an indicator of fecal pollution and the term was often misapplied, and misinterpreted, when applied to water and milk thereby creating confusion.

When the Federal Public Health Service adopted coli-like-forms of bacteria in 1914 as an indicator of fecal contamination in food and water, little information was known about disease causing organisms. There were basically two coli-like forms of interest to the medical and sewage scientists, Bacillus coli and Bacillus aerogenes. Scientists were aware that B. coli was characteristic of fecal origins, while B. aerogenes was rare in feces, but it was the prevailing documented bacteria in soil and grain.

D. H. Bergey, Laboratory of Hygiene, University of Pennsylvania, reported in 1919 that the optimum growth temperature for pathogenic bacteria was 37.5°C. The maximum growth temperature was found to be about 45°C. His focus was on thermophiles which show little or no growth below 40 to

45°C. He found optimum growth for the thermophile Nocardia, both non-spore forming and spore form types, is above 50°C at between 60 and 70°C. Currently we know Nocardia is a pathogenic soil bacteria that causes brain abcesses, mycetoma, pneumonia, and glomerulonephritis. It also causes foaming in activated sludge wastewater treatment plants.

In the 1919 edition of Modern Surgery, Chambers and DaCosta suggested that B. coli may be responsible for appendicitis (inflammation of the appendix), peritonitis (inflammation of wall of the abdomen), inflammation of the genito-urinary tract, pneumonia (inflammation of the lung), inflammation of the intestine (Gastroenteritis) , leptomeningitis (infant meningitis) , perineal abscess (infection of the soft tissues surrounding the anal canal), cholangitis (infection of the bile duct), cholecystitis (inflammation of the gallbladder), myelitis (inflammation of the spinal cord), puerperal fever (childbed fever), wound infections and septicemia (bacteria in the blood). Medical studies in the latter half of the 1900s would confirm their suppositions.

The bacteriological nomenclature of B. coli was changed in 1919 to Escherichia coli. Even though E, coli was known to have pathogenic strains, scientists gave it a harmless reputation as an indicator of fecal pollution because it was easy to grow. As it doubled ever 20 minutes at 37C (98.6F), a colony that could be seen with the naked eye developed overnight.

By 1920, Max Levine, Department of Pathology and Bacteriology, State University of Iowa, concluded scientists must find a way to suppress B. coli and/or B. aerogenes in the test procedures before the true incidence of either could be determined in water or feces. He discovered that the type and ratio of chemicals and dyes could suppress the growth of either one, or both, as well as determine the growth rate. One example given was 65,000 B. coli per C.C. (gram or milliliter) multiplying to 1,500,000 colonies per gram within 48 hours, while B. aerogenes decreased from 2,300 per C.C. to 20 colonies per gram in 24 hours and was non-detectable in 48 hours.

Levine noted there was a significance difference in recommended growth temperature between B. aerogenes at 30°C and B. coli at 40°C. Levine appeared to question the fact that while the maximum temperature for multiplication of B. coli was at 45°C, the Eijkman fermentation test at 46° C was employed for the isolation of B. coli from water. The most important finding of Levine was that while using peptone lactose media, all cultures of B. coli colonies grew extremely well at 43°C within 24 hours. However, 69 percent of the B. coli cultures did not ferment any gas or perhaps only a bubble in 24 hours. The difference for B. aerogenes was even more striking for 20 cultures. Sixteen cultures showed no growth, 2 had slight growth and 2 grew extremely well.

According to Professor Joshua Lederberg, the proto-type laboratory strain of E. coli was isolated from a human at Stanford University in 1922 and eventually designated strain K-12. Barbara J. Bachmann noted in 1972 that K-12 is actually the definitive wild strain which came from a convalescent diphtheria patient. During the intervening 50 years it was used extensively in the teaching laboratories and by scientists working on recombinant experiments thereby creating thousands of K-12 mutants strains. She also reported the creation of X-ray-induced auxotrophic mutants bacteria that would not grow without some added nutrient the parent bacteria did not require. In 1997, EPA stated the clinical parent strain of K-12 had become nonpathogenic:

> "The strain E. coli K-12 is a debilitated strain which does not normally colonize the human intestine. It has also been shown to survive poorly in the environment, has a history of safe commercial use, and is not known to have adverse effects on microorganisms or plants."

However, EPA said some of "Its derivatives are currently used in a large number of industrial applications, including the production of specialty chemicals (e.g., L-aspartic, inosinic, and adenylic acids) and human drugs such as insulin and somatostatin. Further, E. coli can produce a number of specialty chemicals such as enzymes which would be regulated under TSCA [Toxic Substances Control Act]." For the K-12 mutants to do their magic, they must be genetically engineered by the insertion of new genes. When new genes were inserted, scientists also inserted antibiotic genes as markers to prove the successful insertion. The most important observation made by EPA was the fact that "a major shift in nosocomial (hospital acquired) infections from Gram-positive to Gram-negative bacteria occurred in hospital patients during the 1960's and the early 1970's." It has been documented that bacteria are discharged from wastewater treatment plants and may enter the drinking water supply though water treatment plants to cause biofilms in pipes as well as waterborne outbreaks.

By1926, H. Heukelekian was studying Bacillus coli and Bacillus aerogenes in New Jersey activated sewage treatment plants. He noted that the main purpose of a treatment plant was to significantly reduce the number of bacteria in sewage. However, no one understood exactly how that happened. The working theory was that even though intestinal bacteria were reduced, it was "conceivable and probable" that they played an important role in purification of sewage. The cultures used were incubated for 48 hours at 37°C. A special dye was used to suppress colonies from spreading. While Heukelekian found the density of the bacteria varied during the year, his most surprising find was that the reduction of bacteria was because they associated, or attached, to the fine suspended solids (e.g. biofilms). This was demonstrate by centrifuging the treated solids which caused an increase in the number of bacteria. The Water Environment Federation scientists appeared to be shocked when they discovered the same phenomenon 80 years later in 2006 after centrifuging sludge. It would appear Heukelekian was the first to suggested flocs should be added during the treatment process to help draw the fine suspended solids in liquid sewage together in clusters to increase the concentration of solids in sludge. He suggested that B. coli is also responsible for decomposition of carbohydrates, which increases acidity, which decreases the number of B. coli.

Laban Leiter, School of Hygiene and Public Health, Johns Hopkins University, died before his paper on the Eijkman fermentation test was presented at the 1928 meeting of the Society of American Bacteriologists by a colleague, in which he stated the Eijkman test at 46°C was selective for B. coli from fecal contamination by man and other warm blooded animals in water within 24 hours, while other organisms are either inhibited in growth or destroyed. That was a major scientific mistake. We now know the growth of B. coli is inhibited at that temperature. The actually inactivation temperature by moist heat is 121°C (250°F) for at least 15 min or using dry heat 160–170°C (320-338°F) for at least 1 hour. Moreover, Leiter noted that many bacteria from cold blooded animals reacted like B. coli when tested at 37°C, but failed to grow at 46°C. It was Leiter's scientific opinion that these coli-like bacteria from cold blooded animals were of no significance when found in water.

J. W. Brown and C. E Skinner followed up in 1930 with a study comparing B coli recovery by the Eijkman test at 46°C (114.8°F) against the standard test at 37.5°C (98.6°F). It was their belief the number should be the same with both tests. It was also their belief that the coli- aerogenes group had no sanitary significance for determining fecal pollution and was completely suppressed by the Eijkman test temperature. However, they also noted that B.coli could be found in cold blooded animals, soil, and streams with no apparent human pollution. The selling point for the test was that false positives were much rarer with the Eijkman test than with the standard test. They noted several problems with the Eijkman test: 1) Eijkman broth produced fewer colonies than lactose broth; 2) not all B. coli produced gas in 48 hours; 3) some B. coli produced gas in 24 hours but contained few or no living bacteria in 48 hours; 4) other B. coli produced no gas within 24 hours but produced gas within 48 hours; 5) the Eijkman test was expected to give many false negatives; 6) bacteria were quickly killed after gas formation; 7) failure of confirmation at 46°C was either due to strain variation or resistance to the high temperature; 8) it was thought the failure to confirm B. coli giving off gas in the standard test was due to having died because of acidity at the higher temperature; 9) a negative Eijkman test appeared to be of little value. Their final thoughts were with earlier studies which showed only 38.8 per cent of 36 strains and 37 per cent of the 31 strains in fecal material grew at 45°C.

Tonney and Noble, Department of Health, Chicago, noted in 1931 there was still a scientific debate concerning the sanitary significance of B. aerogenes in relationship to B. coli. Their conclusion was that B. aerogenes had no sanitary significance. The main focus of the study was the persistence of B. coli and B. Aerogenes in rotten stumps. Studies had indicated a ratio of B. coli vs B. Aerogenes at 100 to 1, with B. Aerogenes surviving longer in the environment. They also noted a major difference in time vs temperature. In Winter, recovery of surviving organisms was measured in a matter of days. However, when the stumps were spiked in the Spring, bacteria grew during the summer and recovery of surviving organisms was up to 228 days.

In 1935, Maryland's Department of Health concluded that a modified Eijkman test using dextrose at 46°C for B. coli was more selective and efficient for fecal pollution than the standard coil-aerogenes test using lactose as the growth media. C.A Perry notes the Eijkman test was designed to eliminate members of the coli-aerogenes group that were not B. coli. They found that only 11.2% of the lactose fermenting coli-aerogenes group were confirmed as B. coli in an earlier study. The problem they were trying to overcome was that oyster water of "unquestionable purity' for growing oysters far exceed the drinking water standards. While they did prove that more thermotolerant B. coli was confirmed in the Eijkman dextrose test than the lactose test for coli-aerogenes test, they did uncover the illogical claim of safety with the 24 hour test. Some of the coli-like forms of bacteria that ferment dextrose gas in the Eijkman fermentation tubes are slow growing. Out of 422 fermentation tubes showing gas within 48 hours, only 204 were actually confirmed to be B. coli. However, only 62 tubes had gas in them within 24 hours. It took another 24 hours for gas to be fermented in the other 142 tubes. The main point of the study was the Eijkman test was half the work of using the lactose broth for fecal pollution of oysters where the coli-aerogenes group out numbered B. coil by 50 to 100 times. The test missed over two thirds of the coliform bacteria.

By 1937 the term "Escherichia-Aerobacter intermediates" was given to the group of coliform that were somewhat similar to B. coli or B. aerogenes, but could be found in soil water and both warm blooded as well as cold blooded animal feces. These "intermediates" were first reported in 1924.

Philip Carpenter and MacDonald Fulton reported that out of 466 fecal samples, only 90.3% contained E. coli, 46.1% contained Aerobacter and 13.3% contained "intermediates" bacteria. They also reported that one human individual continued to be colonized with intermediates after two years. They concluded the citrate-positive, methyl-red-positive, Voges-Proskauer-negative intermediate (e.g. coliform) group were of sanitary significance.

Leland W. Parr, Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, The George Washington University, referred to the coliform group as the Escherichieae tribe in 1939. Later the group of gram negative bacteria would fall under the heading of the family Entrobacteriaceae. E. coli was thought of as "a primitive aggressive form" of the group from which all other colon bacteria developed. Parr noted there was a normal strain of E. coli and a wild, non-lactose-fermenting E. coli as well as a heat resistant form. The heat resistant form is now referred to as fecal coliform. Of particular interest is the slow fermenters which do not produce gas and/or acid within 24-48 hours. He also points out that a "mutant" or "unstable" form of E. coli may give rise to daughters that would appear to have no relationship to the parent strain and appear to be new species. An example he reported on was two cultures which changed from Escherichia to Aerobacter. There was a scientific debate as to where the evolution was from Escherichia to Aerobacter or the reverse, from A. aerogenes to Escherichia.

Apparently bacterial food poisoning was a major concern back then. Two outbreaks were recorded, 1 in Ohio and 1 in New York. They were attributed to Aerobacter. It caused intestinal infections, urinary tract infections, blood poisoning and soured milk. The coliforms, E. coli and Klebsiella, were considered to be of the most concern for animal disease. Parr thought the coliform group was probable the etiological agents that caused the highly fatal infectious diarrhea of the new-born. He noted that once it invaded a hospital nursery the only way to control it was to close the maternity service. He noted soap doesn't always kill bacteria such as Klebsiella. The overgrowth of Kelbsiella was documented to have caused the explosion of 3 barrels of soap in a military warehouse in Belgium. Furthermore, it had been documented to cause "diseases of the respiratory tract, rhinoscleroma, war wounds, suppuration, meningitis, gaseous emphysema, septicemia, fetid nasal catarrh, infections of the urinary tract, infectious diarrhea of the newborn, and bronchial asthma. Proteus morgani had also been documented "as the etiological agent in summer diarrhea of infants, infectious diarrhea of the new-born, diarrhea and dysentery in adults, infections of the urinary tract, meningitis, chronic discharging wounds, ulcerative colitis, war wounds, fatal septicemia and a paratyphoid like infection." Other coliforms caused "pyelitis, cystitis, cholecystitis, cholangitis, suppuration, septicemia, war wounds, Winckel's disease or hemorrhagic septicemia of the new-born, sepsis neonatorum, infectious diarrhea of the new-born, gastro-enteritis, food poisoning, peritonitis, diarrhea, meningitis, arthritis, intestinal intoxication, gaseous emphysema, and rare cases of infectious dermatitis." He also noted coliform bacteria can cause gas in tissues even though it is generally attributed to Clostridium welchii. We can relate to this as the potentially life-threatening Gas gangrene which causes cell death. It was assumed soil scientists were not interested in the coliform bacteria because many believed, even though they were plentiful in soil, they were not found where there was no animal life. We know that is not true anymore, but the problem is that vectors such as flies play a major role in the spread of coliforms. Not only that but coliform were found in the decaying parts of fruits and vegetables and was/are known to cause soft-rot disease. Parr notes "most raw milk contains coliform bacteria" but the general concern is that they "produce gas and undesirable flavors and odors." Coliform in water was a different story as it was the cause of "Pump infection". In those early days many homes had well pumps. Coliform was

well known to grow on leather washers, wood, swimming pool ropes and it developed slime (biofilms) in pipes. They knew that bacterial slime in the pipe affected the analysis of the water. Also bacterial slime on wood would cause high coliform counts in nearby water. Moreover, it was known that coliform grew well in paper and wood pulp (papermill sludge). What is most interesting is that it was documented that coliform bacteria had a predisposition to "shift" during the testing from a positive to a negative phase in relation to the chemicals normally used. In affect E. coli might show either a positive response or a negative response and not ferment lactose for several days. Parr states, "The significance of these findings for sanitary science is that all of the coliform bacteria must be thought of as possibly fecal in origin."

R.P. Elrod, The Department of Animal and Plant Pathology, The Rockfeller Institute for Medical Research, reported on the Erwinia-coliform relationship within the Enterobacteriaceae family in 1942. Erwinia is a plant pathogen causing soft-rot disease. He was concerned it was so similar E. coli that it might be confused with the coliform group. Other scientists thought it belonged to the colon-typhoid-dysentery group. The Entrobacteriaceae group and coliforms are determined by their reaction to the IMViC tests (Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test) which confirmed Erwinia as an aberrant coliform. By 1967, it was acknowledged as an emerging human pathogen. In 1970, there was a nationwide epidemic of hospital acquired septicemia infection from Erwinia contaminated intravenous fluid products. In 1971, it was documented to cause brain abscesses.

Haskell Tubiash's 1951 Escherichia-Aerobacter density studies in meat curing brine for the United States Department of Agriculture (USDA) found high nitrate levels created false negatives in the coliform test. He discovered that coliforms were growing but failed to produce gas until the brine solution was diluted from full strength in 10 ml test tubes to a brine dilution of 1 ml or 0.1 ml in 10 ml tubes. This finding was of particular importance because many rural drinking water wells contained nitrate levels high enough to cause the false negatives by stopping gas production in the tests. Not only did it stop gas production in the bacteria, but in some cases it stimulated growth of the bacteria. Where this occurred, the rural family was faced with a two-pronged health threat caused by high nitrates: methemoglobinemia (blue baby syndrome) and bacterial infection from contaminated water. Both are baby killers.

Edwards and Ewing's work at the CDC in 1952 focused on serologic typing of the known enteric members of the Enterobacteriaceae family. Salmonella was the most studied. There were over 200 antigenic O and H types. It was acknowledged that most small labs were not capable of typing the individual strains. An unusual aspect was Salmonella's ability to transform from an O to an H type in the laboratory. Scientists were just starting to get an understanding of Shigella. However, little was known about the heat-stable antigens of Salmonella and Shigella. There were 125 O strains of E. coli, but few labs were capable of determining serologic types. Moreover it was difficult to identify the H antigen strains. At that time only two serologic types of E. coli had been identified to cause infantile enteritis. Until this time most Klebsiella capsular strains studied were from the respiratory tract and the skin at the top of the legs and anal area. It was noted that Aerobacter aerogenes and Kelbsiella were identical in the tests. Furthermore, with the advent of antibiotics more Klebsiella were being found in urinary tract infections. Only one new strain in the United States had been found to cause intestinal infections. Thus, there were a total of capsule types known to cause pneumonia, urinary tract infections and intestinal infections. However, knowledge was still lacking for the O antigen type. Moreover, the unknown relationship between Klebsiella slime antigens and

capsular antigens needed to be addressed. They also discussed the paracolon group. This is the intermediate group between E. coli and Klebsiella/Aerobacter that only ferment lactose under prolonged incubation. In effect, they won't show up in the coliform or fecal coliform test. However, they have been a source of infant diarrhea outbreaks in institutions. The paracolon bacteria include the Arizona group some of which takes two weeks to ferment lactose. The Arizona group causes disease in both warm blooded and cold blooded animals. It also causes a high rate of infections and death in fowls as well as humans.

Frances Shattock reviewed why there was not enough information available in 1955 to use serological techniques for the identification and natural classification of bacteria. He noted that the antigenic structure of the Enterobacteriaceae family was being built. An example was the sharing of antigens between E. coli and Klebsiella which indicated not only a close family relationship but in fact a continuous series. Shattock noted serology was a good basis for classifying viral bacteriophages and coli-phages. Coli-phages only infect E. coli. However, there were still problems classifying all the species of the non-coliforms: Streptococcus, streptococci of group D, pneumococci, staphylococci, Clostridium, Pseudomonas aeruginosa, Bacillus, Lactobacillus, viruses, rickettsias and the pleuropneumonia group.

Even in 1957, the Public Health Service was still operating on Christiaan Eijkman's 1904 assumption that only fecal coliforms from warm blooded animals could grow at 46°C (114.8°F) and produce gas. It was assumed that Joseph Leiter was right in 1929 when he suggested that the high temperature and positive indole production was specific for E. coli. However, H. F. Clark and his associates at Robert A. Taft Sanitary Engineering Center in Cincinnati recognized in the first of two studies on Coliforms incubated at 43°C that many claims had been made for a better suited procedure for E. coli growth was available, which also suppressed growth of non-coliform gram negative bacteria. All other major pathogens are ignored. The main criticism of all tests was the possible failure of E. coli growth due to minimum numbers planted on the media plates in the Most Probable Number (MPN) method. In their survey of surface water from 14 geographical areas of the United States Clark and his associates found the standard coliform test (IMViC) reactions create a large number of false positives. In this instance a wide variance existed between coliform flora in the different locations. However, when combined with the reaction in boric acid lactose broth (BALB) many false positives could be eliminated which would be sufficient for survey works.

The second study on Fecal coliforms by the Public Health Service was published in 1958. E.E. Geldreich and associates focused on E. coli as a more accurate indicator of fecal pollution for water, milk, and food products when incubated at 45°C for 24 hours in EC (E. coli) medium. According to the authors:

> "The coliform group of bacteria for this investigation was defined as aerobic and facultative anaerobic gram negative, nonsporeforming bacteria which fermented lactose with gas formation in 48 hr, or less, when incubated at 35°C (Standard Methods for the Examination of Water, Sewage, and Industrial Wastes. APHA, 1955a)."

Two types of E. coli (fecal coliform) were found. One was indole positive and one was indole negative. A total of 4,436 surface water samples were collected from 14 treatment plants. Only 1,358 samples (83.7%) included E. coli with a positive reaction in EC medium at 45°C and were concluded to be fecal bacteria. However, 21.8% of the E. coli samples were actually indole

negative. Ten other coliforms showed a positive reaction in EC medium in 348 samples (7.8%) at 45°C, but were concluded to be nonfecal bacteria. The Public Health Service was not comfortable with relying on the two tests (IMViC reactions and growth at elevated temperatures) to determine fecal pollution. It was suggested that other considerations should also be used to verify fecal pollutions along with the two tests. They were not sure of the sanitary significance of those bacteria not considered to be fecal coliforms. Their conclusion was that the EC medium at 45°C was suitable for enumeration of E. coli in field work, but it was necessary to further verify fecal pollution.

It wasn't until 1980 that Jean MacFadden consolidated some of the information on indole positive bacteria such as: Aeromonas hydrophilia, Aeromonas punctata, Bacillus alvei, most Citrobacter sp., Edwardsiella sp., Escherichia coli, Flavobacterium sp., Haemophilus influenzae, most Proteus sp. (not P. mirabilis), Plesiomonas shigelloides, Pasturella multocida, Pasturella pneumotropica, Streptococcus faecalis, and Vibrio sp. The indole negative bacteria she found included: Actinobacillus spp., Aeromonas salmonicida, Alcaligenes sp., most Bacillus sp., Bordtella sp., Enterobacter sp., Lactobasillus spp., most Haemophilus sp., most Klebsiella sp., Neisseria sp., Pasturella haemolytica, Pasturella ureae, Proteus mirabilis, Pseudomonas sp., Salmonella sp., Serratia sp., Yersinia sp.

The Public Health Service was still trying to figure out the sanitary significance of fecal, vegetable, and soil coliforms in 1964. They did find there was a difference in the production of hydrogen and carbon dioxide from glucose among bacteria feed in feces and grain. The two fecal coliforms are an example. E. coli ferment glucose in fecal material to produce equal amounts of hydrogen and carbon dioxide (1:1) while Aerobacter aerogenes produced twice as much hydrogen and carbon dioxide (1:2). However, in grain coliforms produced 2 to 3 (1:2 - 1:3) times as much hydrogen and carbon dioxide. It was assumed this was a good way to tell the difference between coliforms from warm blooded animals and those from grain. The sanitary significance of the IMViC classification for coliform was unclear for the relationship between fecal, soil, vegetation, and other bacteria. Furthermore, it was understood that IMViC may undergo changes in artificial environments and wastes waters. While it was clear to the authors, Eijkman's elevated temperature test at 46°C separated fecal coliforms from warm blooded animals from coliforms from other sources, the current recommendation of using an incubation temperature of 43°C for 48 hours with the addition of boric acid suppressed aerobacter and the intermediate paracolon group was unacceptable. It was suggested that an incubation temperature of 44.5°C in EC media was more acceptable. It was their belief that a small number of fecal coliform would be excluded and a small number of non-fecal coliforms would be included. More over the test should only be used as a confirmatory procedure with the stipulation that a positive reaction from unpolluted soil must be considered nonfecal coliforms. However, they found thermotolerant fecal coliforms from humans, cows, sheep, pigs, chickens, ducks and turkeys. They were also found in feed lots, soil with no known fecal pollution, soil flooded with domestic sewage, on plants as well as on insects. There final warning conclusion was:

> "Because no satisfactory method is currently available for differentiating fecal coliform organisms from human and other animal origin, it is necessary to consider all fecal coliform organisms as indicative of dangerous contamination."

There was a major problem with Eijkman's elevated temperature test as pointed out by Eliora Z. Ron and M. Shani in 1971. When the incubation temperature is increased from 42 to 45°C, the

activity of certain enzymes are suddenly lowered causing a decrease in cell growth capabilities. This was attributed to methionine starvation. However, it doesn't indicate the cells are injured as growth returns to normal when the incubation temperature is lowered to 37°C. When the temperature was raised to 47°C for 10 minutes, the decreased growth capabilities were not completely reversible as less than 10% of the enzyme growth capability was restored.

Charles Hendricks' 1972 EPA funded study on Heterotrophic, enteric, and coliform bacterial growth in river water gives a hint of the coming confusion concerning water, food and sewage testing. All three classifications include the etioloc pathogens listed here. Hendricks mentioned "natural heterotrophic bacterial populations" but never fully explained that this includes all pathogenic and nonpathogenic bacteria as well as fungi that obtain energy from oxidation of organic compounds. Among these are the six laboratory culture strains of enteric bacteria used in the study: Escherichia coli, Enterobacter aerogenes, Proteus, Arizona, Salmonella, and Shigella spp. Culture generation time in the study ranged from 33.3 to 116 hours at the maximum temperature growth rate of 30°C. Of these, only the fast growing E. coli (34.5 hours) and E. aerogenes (33.3 hours) were mentioned as coliforms and fecal coliforms (i.e., thermotolerant coliforms) indicators of fecal pollution, when cultures were incubated at 30°C and 44.5°C. Hendricks acknowledged all six enteric bacteria were members of the Enterobacteriaceae family but stated others such as Salmonella, Shigella, and Arizona could also produce serious intestinal diseases. These are also referred to in the study as coliforms when incubated at 30°C. He determined that it took 2 to 3 times as long to grow a culture of these bacteria as it did for E. coli and E. aerogenes. Enteric bacteria found in river water normally existing under starving conditions required a culture generation time of 116 hours. An unusual finding was that enteric bacteria from river water grew in a slime layer on glass (e.g. biofilms) and sloughed off about every 100 hours whereas the stock laboratory culture strains did not. The essence of the study was that neither the stock enteric laboratory strains or the enteric river water strains would grow in autoclaved river water taken from above two of three sewage treatment plants, while all grew in autoclaved river water taken 750 meters below the sewage treatment plants outfall. While the temperature is of the most interest for the test results, Hendricks states:

> "Data reported in this study certainly cannot be extrapolated directly to the natural aquatic environment where one could say unequivocally that enteric bacteria, including pathogens,are capable of growth in fresh water."

B. J. Dutka and associates found in 1979 that the fecal coliform and E. coli estimates were just the tip of the iceberg. E. coli, Klebsiella and Enterobacter were obtained from hospital patients and tested at temperatures of 35°, 41.5°, 43°, 44.5°, and 35°C for 4 h followed by 18 h at 44.5°C. Only about 5% of the bacteria population incubated at 44.5°C were recovered while 100% of the population was recovered at 35°C.

Indicator bacteria (e.g. tracers) have been used since the first part of the 1900s to trace sewage pollution according to a 1980 study by L.W. Sinton, Water and Soil Science Centre (MWD) Christchurch, NZ. He investigated to mutant nonpathogenic antibiotic resistant derivatives of K12, the lactose negative E. coli J6-2 and the lactose positive E. coli PB 922. He noted Serratia marcescens was used as a tracer in 1909 and again in 1920. Serratia marcescens, Chromobacterium violaceum and Bacillus subtilis were used in 1957. Bacterophage was use in 1974 and followed by Bakers yeast (Saccharomyces cercevisiae) in 1978. The advantage of

antibiotic resistant S. marcescens was established in 1956 and B. subtilis was added to the list in 1969. In 1978 antibiotic resistant E. coli and Streptococcus faecalis was being used. The problem was that S. marcescens had been implicated in blood poisoning and other infections. B. subtilis had been implicated in blood poisoning, eye infections, urinary tract infections, pneumonia, and other health effects. Sinton also noted E. coli was the most frequent cause of hospital infections.

In 1980, University of Texas researchers Donald Dudley and associates appears to be the only group that attempted to screen for potential etiologic pathogens in an effort to establish the risk of treated sludge land application. Their conclusion was that primary or digested sludge should not be used on agricultural or recreational land, especially where food crops are grown, unless the crops undergo heat processing, or unless sludge undergoes composting, irradiation, or pasteurization. Their conclusion was based on finding 17 named members of the Enterobacteriaceae family (coliform and fecal coliform), Salmonella sp., Shigella sp., Klebsiella sp., Citrobacter diversus subsp. Levinae, C. freundii, Enterobacter aerogenes, E. agglomerans, E. cloacae, E. sakazakii, Escherichia coli, Hafnia alvei, Klebsiella oxytoca, K. ozaenae, K. pneumoniae, Proteus morganii, Serratia liquefaciens, S. marcescens, S. rubidaea, Yersinia enterocolitica, Y. ruckeri. They also found 17 named members of the Oxidase-positive, gram-negative enteric bacteria: Achromobacter sp., A. xylosoxidans, Acinetobacter calcoaceticus var. lwoffi, Aeromonas hydrophila, Alcaligenes sp., Bordetella bronchiseptica, CDC Group V E-1, Flavobacterium odoratum, Pseudomonas aeruginosa, P. cepacia, P. fluorescens, P. maltophilia, P. paucimobilis, P. putida, P. putrefaciens, P. stutzeri, Vibrio alginolyticus. Plus,the following: streptococci, Fluorescent pseudomonads, Staphylococcus sp., Clostridium perfringens (Vegetative (at room temp) & Sporulated (at 80°C)), Mycobacterium sp.. The implication of the study was that coliform and fecal coliform was something different from the Enterobacteriaceae. They noted Salmonella (a coliform) had been found in 90% of sludges and it survived up to 72 weeks in land applied sludge. Mycobacterium tuberculosis survived up to 15 months in sludge drying beds. Clostridium perfringens was found to be concentrated in sludge. Unfortunately, Shigella was very difficult to isolate from sludge. Klebsiella pneumoniae was found in large numbers while only low levels of Staphylococcus aureus where enumerated. With the exception of the fecal coliform and Salmonella tests, all test were run at 37°C (98.6°F) for 18 to 48 hours. They found enriched test media was needed to obtain best results and high temperatures decreased survival. Plus, not all bacteria could be enumerated in 48 hours such as Mycobacterium tuberculosis which took weeks to create colonies. Most importantly, it was noted fecal indicators could be recovered for months after dewatered sludge was applied to land. Dudley warned, "Pathogens removed through wastewater treatment should not be reintroduced into a population via new reservoirs that may be established by irresponsible land management of application sites."

By 1981 the most-probable-number (MPN) test procedures for coliform aerobic and facultatively anaerobic bacteria that ferment glucose to produce gas and/or acid within 48 hours had been adopted as the gold standard for determining fecal pollution in water and food. However, David Hussong and associates at the University of Maryland and USDA were concerned about the types of bacteria causing high numbers of false-positive coliform test results. The accuracy of the test was also questioned due to "injured" and atypical coliforms. Samples of water, shellfish, and sediment were collected from two shellfish harvesting areas of Chesapeake Bay. The coliform procedure used lactose broth (LB) as a medium with brilliant green bile (BGB)broth as a confirmatory medium for fecal coliform. Both mediums used an incubation temperature of 35°C for 24-72 hours. A total of 588 LB positive, BLB negative colonies of bacteria were incubated up to 10

days for clasification. The positive LB samples included Enterobacteriaceae (49%), Aeromonas spp. (25%), and Bacillus spp. (16%). The Enterobacteriaceae family included: Enterobacter (32%), Hafnia (19%), Klebsiella (9.7%), Proteus (9%), Serratia (17%), Escherichia (3.2%), and Erwinia (9.7%).Most of the bacteria identified from the negative BGB fecal coliform test were from the family Enterobacteriaceae (69%); which included Serratia liquefaciens (22%), Enterobacter spp. (21%), Erwinia herbicola (12%), Hafnia alvei (8%), Proteus morganii (3%), and Klebsiella spp. (3%). Aeromonas spp. (28%) was also found as were Pseudomonas maltophilia (2%) and Bacillus strains (1%).

Jose L. Alonso and associates found in their 1999 study on recovery of E. coli and therotolerant bacteria in Chromogenic Medium Incubated at 41 and 44.5°C, that E. coli was the only bacteria for which standard data existed. They restated the belief of Leclerc et al. that the only coliforms of fecal origin and their frequency of recovery were: E. coli, 100%; Citrobacter diversus, 70%; Citrobacter amalonaticus, 70%; Citrobacter freundii, 70%; Klebsiella pneumoniae, 49%; Klebsiella oxytoca, 49%; Enterobacter cloacae, 9%; and Enterobacter aerogenes, 9%. The nonfecal coliforms they believed were: Klebsiella trevisamii, Enterobacter agglomerans, E. gergoviae, E. sakazakii, Hafnia alvei, Serratia marcescens, S. liquefaciens, S. marinorubra, and S. odorifera. They added sodium pyruvate to the medium to aid in the recovery of chlorine "injured" bacteria and found the optimum recovery incubation temperature to be 41°C. In their tests incubation at 44.5°C inhibited the growth of all nonfecal coliforms and many fecal coliforms. It was concluded the main source of false-positives where the incubation temperature was 35-37°C was Vibrio and Aeromonas sp, which could not grow at 41°C. They and others suggested the term fecal coliforms should be removed from microbiology literature and replaced with the term thermotolerant bacteria.

The World Health Organization (WHO) outlined some of the problems of using coliforms as fecal indicators in 2001. Among the problems was that some indicators were pathogens and even E. coli was problematic to detect due to the potential to form viable but nonculturable cells. WHO notes the environmental growth of thermophilic (fecal coliforms) such as E. coli and Klebsiella have been a concern of sanitary engineers since the 1930s. It was also noted that some coliforms, including E. coli, were ignored because they fail to produce gas from lactose or were indole negative when incubated at 44.5°C. Moreover, WHO states:

> "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)" and "water regulatory agencies have yet to come to terms with the inherent problems resulting from reliance on faecal indicator bacteria as currently determined."

The Alice Minter Trust farm soil tests matches WHO's observation that a small fraction of the thermotolerant fecal coliform bacteria are enumerated in the tests.
E. coli >800,000 -- fecal coliform 3,000 = 0.00375
Salmonella >800,000 -- fecal coliform 9,000 = 0.01125

EPA has developed a complicated test to determine one form of thermotolerant bacteria in liquid or semi-liquid sewage sludge and converted those numbers to a dryweight. In the 1989 proposed rule EPA stated it could not use the term biosolids. By 1993 EPA mentioned biosolids 4 times in the preamble with the comment that "wastewater residuals (sewage sludge is also often referred to as

“biosolids”)." While EPA's Part 503 Sludge Rule does not use the term biosolids, the term "sewage sludge (Biosolids)" is only used three times in the 51 page test procedure. The term biosolids is used 65 times. The test is about complex calculations with charts, counting bacteria colonies at the end of the test and reporting them as the most probable number or number of colony forming units at the beginning of the test. This assumes that by some type of Sludge Magic the bulk sludge bacteria are heat inhibited and stop growing when the samples are taken. More important, the positive and negative control bacteria used are nonpathogenic laboratory cultures which do not react like real fecal bacteria. In effect, What EPA is saying is that no matter how many species of pathogenic bacteria are in your feces that grow well and produce gas at 37°C (98.6°F), it is not going to admit they are fecal unless they grow at the elevated temperature of 44.5°C (112.1°F) and produce gas. On the other hand laboratory technicians must assume any bacteria in sludge are pathogens.

According to EPA's 2006 test method 1681 for fecal coliform:

> "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 ± 2 hours in A-1 broth after incubation at 44.5°C ± 0.2°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."

Moreover, EPA warns:

> "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."
>
> WARNING: The drying oven should be contained in a hood or be vented. Significant laboratory contamination may result from drying a heavily contaminated sample.
>
> 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.
>
> "Obtain a stock culture of E. coli (e.g., ATCC # 25922) as a positive control for A-1. Note: ATCC recommends that no more than 5 transfers be made before returning to the original culture. This will minimize the chance of contamination during transfers and genetic shift of the culture." [Nonpathogenic clinical isolate FDA strain Seattle 1946, with incubation Temperature: 37.0°C]
> "Obtain a stock culture of Enterobacter aerogenes (e.g., ATCC # 13048) as a negative control for A-1." [Nonpathogenic, sputum, South Carolina Dept. of Health and Environmental Control with incubation Temperature: 30.0°C]
>
> Since sample fecal coliform densities are expected to be variable, it is recommended that at least seven biosolid samples be analyzed using this method. The geometric mean fecal coliform density of the seven biosolids samples should not exceed 2 × 106 MPN/g of total solids (dryweight basis) to qualify as Class B biosolids. Although there is not a specific number of samples required for Class A biosolids, it is recommended that a sampling event extend over two weeks and that at least seven samples be

> collected and determined to be below 1,000 MPN/g of total solids (dry weight basis) to qualify as Class A biosolids.
>
> Due to the extreme variability in the solid content of biosolids, fecal coliform results from biosolid samples are reported as MPN/g total solids (dry weight basis)"

EPA has also approved 10 enzyme based tests for the presence or absence of total coliform organisms and E. coli in water. The premise of the tests are that they suppress all bacteria except the coliforms. Jeremy Olstadt and associates at the Water Bacteriology Department, Wisconsin State Laboratory of Hygiene, compared the test in 2007. However, the implication of study is that only four gram negative fermentative Enterobacteriaceae members make up the total coliforms: E. coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., and Serratia spp.. It is most interesting to see that only the nonEnterobacteriaceae gram negative fermentative Aeromonas spp. was used to confirm the tests actually suppressed other pathogens. There appears to be no concern for nonfermentative pathogenic bacteria in water. The primary finding of the study was that the tests did not always work as claimed for the approved purpose. This is particularly important based on the finding that drinking water outbreaks of gastrointestinal disease has become a major problem in recent years. They also note CDC's finding that contaminated ground water accounted for about 70% of the outbreaks between 1987 and 1997.

The Community College of Baltimore County Biology 230 Laboratory Manual 12 for 2009 identifies Enterobacteriaceae as fermentative, gram-negative, enteric bacilli rather than coliforms. The more important common clinical members are Citrobacter, Edwardsiella, Enterobacter, Escherichia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Serratia, Shigella, Yersinia.

Svenja Lüders and associates confirmed Eliora Z. Ron and M. Shani's 1971 study on methionine starvation of E. coli at high temperatures in 2009. They note the normal range of E. coli growth is between 21°C to 37°C. Higher temperatures decreases enzyme activity with a resulting decline in methionine which inhibits growth between 40-45°C, and stops growth at 45°C.

Rather than having to explain what the coli-like-forms of bacteria were, and the diseases they cause through food and water, federal regulators simply shortened the term to coliforms and claim they do not cause disease in healthy individuals. To top that off EPA adopted the high temperature test for E. coli which inhibits the growth of E. coli and eliminates the growth of other Enterobacteriaceae gram negative pathogens as well other gram negative pathogenic bacteria that would give a false positive in the test. The tests ignore eight other families of bacteria: 1) Nonenterobacteriaceae fermentative gram negative bacteria; 2) Nonenterobacteriaceae nonfermentative gram negative bacteria; 3) Gram-positive Bacilli; 4) Gram-Positive Cocci; 5) Gram-Negative Cocci; 6) Gram-Negative Fastidious Coccobacilli; 7) Mycoplasma (Pleuropneumonia-Like Organisms; 8) Treponemataceae (Spiral Organisms) as well as viruses, helminths, protoza, Fungi, yeast and rickisetta. The result has been the explosion of foodborne illnesses, health related costs and deaths leading to food safety laws aimed at farmers who believed the pathogen contaminated sludge and reclaimed water was safe based on these tests. This scientific con game by EPA, FDA, USDA, state regulators, laboratories, water and sewage scientists is a crime against humanity. While some scientists are speaking out, others appear to be afraid to speak out due to peer pressure or the loss of funding and/or employment. They all know the purpose is to transfer liability from municipalities, states and the federal government to the general public.

References

Alice Minter Trust, Soil Test Report Comparisons, QWAL Laboratories, Inc. http://thewatchers.us/pathogens/test-comparison.html

Allen, Mary Belle, The Thermophilic Aerobic Sporeforming Bacteria, Bacteriol Rev. 1953 June; 17 (2): 125–173, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC180763/

Alonso, Jose L. 1,* Soriano, Adela 2, Carbajo, Oscar 3, Amoros, Immaculada 1, and Garelick, Hemda 3, Comparison and Recovery of Escherichia coli and Thermotolerant Coliforms in Water with a Chromogenic Medium Incubated at 41 and 44.5°C, Instituto de Hidrologia y Medio Natural, Universidad Politecnica, 46022 Valencia,1 Gamaser S.L., 46014 Valencia,2 Spain, and School of Health Biological and Environmental Sciences, Middlesex University, London N11 2NQ, United Kingdom3, Applied and Environmental Microbiology, August 1999, p. 3746-3749, Vol. 65, No. 8 http://aem.asm.org/cgi/content/full/65/8/3746

Bachmann, Barbara J., Pedigrees of Some Mutant Strains of Escherichia coli K-12, Bacteriological Reviews, Dec. 1972,Vol. 36, No. 4, p. 525-557, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC408331/pdf/bactrev00201-0120.pdf

Bergey, D.H., Thermophilic Bacteria, Laboratory of Hygiene, University of Pennsylvania, JOURNAL OF BACTERIOLOGY, VOL. IV, NO. 4, 1919, http://thewatchers.us/PDF_files/copy_of_301facultativethermophiles.pdf

BROWN,J. W. AND SKINNER, C. E., IS THE EIJKMAN TEST AN AID IN THE DETECTION OF FECAL POLLUTION OF WATER?, Department of Bacteriology and Immunology, University of Minnesota, 1930, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC375111/pdf/jbacter00864-0053.pdf

Bynum, James W., Sludge Disposal: Sanitary Landfill-Open Dump-Superfund Site?, Conference Proceeding - New Mexico Governors Conference on the Environment, Vol, 2., New Mexico Environmental Department, February 1993, http://thewatchers.us/New-Mexico-paper.html

Carpenter, Philip L., Fulton, MacDonald, Escherichia-Aerobacter Intermediates from Human Feces, Iowa State College, Ames, Iowa;and School of Medicine, St. Louis University, St. Louis, Mo., American Journal of Public Health, Aug., 1937 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1563268/pdf/amjphnation01049-0092.pdf

CLARK,H. F., GELDREICH,E. E., KABLER,P. W., BORDNER,R. H., HUFF,C. B., The Coliform Group, I. The Boric Acid Lactose Broth Reaction of Coliform IMViC Types, Robert A. Taft Sanitary Engineering Center, Public Health Service, Department of Health, Education, and Welfare, Cincinnati, Ohio, 1957, http://aem.asm.org/cgi/reprint/5/6/396.pdf

Community College of Baltimore County, LAB 12: ISOLATION AND IDENTIFICATION OF ENTEROBACTERIACEAE AND PSEUDOMONAS, PART 1, http://student.ccbcmd.edu/courses/bio141/labmanua/lab12/lab12.html

Dudley,Donald J., et al, Enumeration of Potentially Pathogenic Bacteria from Sewage Sludges,

Center for Applied Research and Technology, The University of Texas at San Antonio, APPLIED AND ENVIRONMENTAL MICROBIOLOGY,Vol. 39, No. 1, Jan. 1980, p. 118-126, http://thewatchers.us/PDF/1980-pathogen-study.pdf

Dutka, B.J., Kuchma, S., Kwan, K.K., Fecal coliform and E. coli estimates, tip of the iceberg, Water, Air, & Soil Pollution, Volume 11, Number 3, 349-362, http://www.springerlink.com/content/vr0uq8q480078448/

Edwards, P.R., Ewing, W.H., The Status of Serologic Typing in the Family Enterobacteriaceae, Bacteriologist and Scientist (R), Communicable Disease Center, Public Health Service, Atlanta, AMERICAN JOURNAL OF PUBLIC HEALTH, 1952, http://ajph.aphapublications.org/cgi/reprint/42/6/665

ELROD, R.P., THE ERWINIA-COLIFORM RELATIONSHIP, J. Bacteriol. Elrod 44 (4): 433, http://jb.asm.org/cgi/reprint/44/4/433

EPA, 21 Cancer causing agents in sludge. Federal Register / Vol. 54, No. 23 / Monday, February 6, 1989 / Proposed Rules p. 5777, http://deadlydeceit.com/1989_503_cancer_list.html

EPA, Gerba, Charles, Development of a Qualitative Pathogen Risk Assessment Methodology for Municipal Sludge Landfilling, EPA/600/6-88/006, 1986, http://nepis.epa.gov/Exe/ZyNET.exe/30000YK2.txt?ZyActionD=ZyDocument&Client=EPA&Index=1981%20Thru%201985%7C2000%20Thru%202005%7C1976%20Thru%201980%7C1986%20Thru%201990%7CPrior%20to%201976&Docs=&Query=600688006%20&Time=&EndTime=&SearchMethod=2&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C86THRU90%5CTXT%5C00000002%5C30000YK2.txt&User=ANONYMOUS&Password=anonymous&SortMethod=-%7Ch&MaximumDocuments=15&FuzzyDegree=0&ImageQuality=r85g16/r85g16/x150y150g16/i500&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x#

EPA, TWENTY-
FIVE PRIMARY PATHOGENS ASSOCIATED WITH A HIGH INCIDENCE OF DISEASE IN SEWAGE SLUDGE, Federal Register / Vol. 54, No. 23 / Monday, February 6, 1989 / Proposed Rules p. 5829, http://deadlydeceit.com/1989_503_pathogens.html

EPA, Escherichia coli K-12 Derivatives Final Risk Assessment, Biotechnology Program Under the Toxc Substances Control Act (TSCA), 1997, http://www.epa.gov/oppt/biotech/pubs/fra/fra004.htm

EPA, Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using A-1 medium, EPA-821-R-06-013, 2006, http://water.epa.gov/scitech/swguidance/methods/bioindicators/upload/2008_11_25_methods_method_biological_1681_1.pdf

EPA, 503 Sludge Rules, 40 CFR Part 257 et a!.Standards for the Use or Disposal of Sewage Sludge; Final, Federal Register / Vol. 58, No. 32 / Friday, February 19, 1993 / Rules and

Regulations, http://thewatchers.us/PDF_files/1993-503-searchable.pdf

EPA, A Guide to the Biosolids Risk Assessments for the EPA Part 503 Rule, Chapter 6, EPA832-B-93-005, 1995, http://deadlydeceit.com/503-Riskassessment.html

FDA, Bacteriological Analytical Manual, Chapter 4, Enumeration of Escherichia coli and the Coliform Bacteria, 2010, http://www.fda.gov/food/scienceresearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm064948.htm

Geldreich, E. E., Clark, H. F., Kabler, P. W., Huff, C. B., Bordner, R. H., The Coliform Group, II. Reactions in EC Medium at 45°C, Robert A. Taft Sanitary Engineering Center, Public Health Service, Department of Health, Education, and Welfare, Cincinnati, 1958, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1057428/pdf/applmicro00319-0063.pdf

Harrison, E.Z., McBride, M.B., Bouldin, D.R., A Case for Caution, 'Land application of sewage sludges: an appraisal of the US regulations', Cornell Waste Management Institute, Int. J. Environment and Pollution, Vol. 11, No. 1, pp.1–36, 1999. http://cwmi.css.cornell.edu/PDFS/LandApp.pdf

Hendricks, Charles W., Enteric Bacterial Growth Rates in River Water, Department of Microbiology, University of Georgia, Athens, APPLIED MICROBIOLOGY,Vol. 24, No. 2, Aug. 1972, p. 168-174, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC380575/pdf/applmicro00049-0026.pdf

Heukelekian, H., THE FATE OF B. COLI AND B. AEROGENES IN SEWAGE PURIFICATION, Paper No. 325 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Sewage Disposal, 1926, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC374946/pdf/jbacter00899-0057.pdf

Hussong, David., Damarr, John M., Weiner, Ronald M., Colwell, Rita R., Bacteria Associated with False-Positive Most-Probable-Number Coliform Test Results for Shellfish and Estuaries, Department of Microbiology, University ofMaryland, College Park, Applied and Environmental Microbiology,Vol. 41, No. 1, Jan. 1981, p. 35-45, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC243637/pdf/aem00194-0055.pdf

Jackson, Daniel D., Classification of the B. Coli Group, Read before the Laboratory Section of the American Public Health Association, Milwaukee, September, 1910.` http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2218909/pdf/japha00012-0062.pdf

Kabler, P.W., Clark, H. F., Geldreich, E. E. , SANITARY SIGNIFICANCE OF COLIFORM AND FECAL COLIFORM ORGANISMS IN SURFACE WATER,This paper was presented at the lnternational Conference on Global Impacts of Applied Microbiology at Stockholm, Sweden, August 1, 1963, Public Health Reports, Vol. 79, No. 1,p. 48, January 1964

Lederberg, Joshua, E. coli K-12, The Rockefeller University,MICROBIOLOGY TODAY VOL 31/AUG04, http://www.sgm.ac.uk/pubs/micro_today/pdf/080402.pdf

Leiter, Laban W., The Eijkman Fermentation Test as an Aid in the Detection of Fecal

Organisms in Water, Presented by William W. Ford, School of Hygiene and Public Health, Johns Hopkins University, Thirtieth Annual Meeting of the Society of American Bacteriologists,1928, Journal of Bacterology, Vol. XVII, No. 1, http://thewatchers.us/EPA/1928-E-coli.pdf

LEVINE,Max, NOTES ON BACT. COLI AND BACT. AEROGENES, From the Department of Pathology and Bacteriology, State University of Iowa,Read before Laboratory Section, American Public Health Association, at San Francisco, Cal., September 16, 1920. Am J Public Health (N Y). 1921 January; 11(1): 21–23
http://thewatchers.us/EPA/2/1921--E-coli-Enterbactor.pdf

Lewis, David L.2, Gattie, David K. 1, A High-Level Disinfection Standard for Land-Applied Sewage Sludges (Biosolids), 1 Department of Biological and Agricultural Engineering, and, 2 Department of Marine Sciences, University of Georgia, Athens, 2003, Environ Health Perspect 112:126-131.

Lewis, David L. 1,2 , Gattie, David K.3 , Novak, Marc E. 2 , Sanchez, Susan 4, Pumphrey, Charles 5, Interactions of pathogens and irritant chemicals in land-applied sewage sludges (biosolids), 1 US Environmental Protection Agency, National Exposure Research Laboratory, Athens, GA, USA
2 Departments of Marine Sciences University of Georgia, Athens, GA, USA
3 Biological & Agricultural Engineering University of Georgia, Athens, GA, USA
4 Medical Microbiology University of Georgia, Athens, GA, USA
5 Prime Care of Sun City, Menifee, CA
BMC Public Health 2002, 2:11, http://www.biomedcentral.com/1471-2458/2/11/qc

Lewis, David, Sludge Magic at EPA, The Journal of Commerce; January 27, 1999, http://www.deadlydeceit.com/SludgeMagic.html

Lüders, Svenja, Fallet, Claas, Franco-Lara, Ezequiel, Proteome analysis of the Escherichia coli heat shock response under steady-state conditions, Institute of Biochemical Engineering, Technische Universität Braunschweig, Gausstrasse 17, 38106 Braunschweig, Proteome Science 2009, 7:36, http://www.proteomesci.com/content/7/1/36

MacFaddin, Jean F. "Biochemical Tests for Identification of Medical Bacteria." Williams & Wilkins, 1980, pp 173 - 183. http://openlibrary.
org/books/OL7719402M/Biochemical_Tests_for_Identification_of_Medical_Bacteria

Markova N, Slavchev G, Michailova L, Jourdanova M. Survival of Escherichia coli under lethal heat stress by L-form conversion. Int J Biol Sci 2010; 6:303-315. http://www.biolsci.org/v06p0303.htm

McCampbell, Eugene F., The Public Drinking Cup. Bacteriological Report, The Ohio Naturalist. [Vol. VII, No. 5, March, 1907.], https://kb.osu.edu/dspace/bitstream/1811/1559/1/V07N05_105.pdf

McDaniel, T. K. and Kaper, J. B. (1997), A cloned pathogenicity island from enteropathogenic Escherichia coli confers the attaching and effacing phenotype on E. coli K-12. Molecular Microbiology, 23: 399–407. http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2958.1997.2311591.x/abstract

New York Times, UNLAWFUL TO DRINK FROM A PUBLIC CUP, 1911,

http://query.nytimes.com/mem/archive-free/pdf?
_r=1&res=9D00E5D71531E233A25752C0A9669D946096D6CF

Olstadt, Jeremy, Schauer, James Jay, Standridge, Jon, and Kluender, Sharon, Comparison of total coliform/E.coli tests, Journal of Water and Health, 05.2, 2007, http://www.iwaponline.com/jwh/005/0267/0050267.pdf

Parr, Leland W., COLIFORM BACTERIA, Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, The George Washington University, Washington, D. C., 1939, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC440831/pdf/bactrev00151-0003.pdf

Peleg, Anton Y., Hooper, David C., Hospital-Acquired Infections Due to Gram-Negative Bacteria, n engl j med 362;19 nejm.org may 13, 2010, http://www.google.com/url?sa=t&source=web&cd=10&ved=0CD4QFjAJ&url=http%3A%2F%2Fxa.yimg.com%2Fkq%2Fgroups%2F16063327%2F586275545%2Fname%2FPeleg%2C%2BHooper%2B-%2B2010%2B-%2BHospital-Acquired%2BInfections%2BDue%2Bto%2BGram-Negative%2BBacteria.pdf&rct=j&q=gram%20negative%20bacteria%2050%25%20hospital%20acquired%20infections&ei=hOpdTPrOKcP_lgfH2OSZCA&usg=AFQjCNGAOZ7oc9xnwLsngmJvMAUlvQMeDg&c
ad=rjt

Perry, C.A., Hajna, A.A., Routine Use of a Modified Eijkman Medium in the Examination of Oysters, Crabmeat, and Other Substances, Bureau of Bacteriology, Maryland State Department of Health, Baltimore,Am J Public Health Nations Health. 1935 June; 25(6): 720–724, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1558997/

Portland, Oregon, http://www.portlandonline.com/auditor/index.cfm?&a=21721&c=28822

Ron, Eliora Z., Shani, M., Growth Rate of Escherichia coli at Elevated Temperatures: Reversible Inhibition of Homoserine Trans-Succinylase, JOURNAL OF BACTERIOLOGY, Vol. 107, No. 2, Aug. 1971, p. 397-400, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC246938/pdf/jbacter00369-0023.pdf

Savage, William G., The Pathogenicity of B. coli in Relation to the Bacteriological Examination of Water, Journal of Hygiene, Vol. 3, No.3, July, 1903, pp. 388-399
Shattock, P. M. Frances, The Use of Serology in the Classification of Micro -organisms, J . gen. Microbial. 12, 367-374, 1955, http://mic.sgmjournals.org/cgi/reprint/12/2/367.pdf

Sinton, L.W. Two Antibiotic-Resistant Strains of Escherichia coli for Tracing the Movement of Sewage in Groundwater, 1980, Water and Soil Science Centre, MWD, Christchurch, http://www.hydrologynz.org.nz/downloads/JoHNZ_1980_v19_2_Sinton.pdf

Snow, John, Dr., UCLA, Department of Epidemiology, School of Public Health, http://www.ph.ucla.edu/epi/snow.html

Tonney, Fred 0., Noble, Ralph E., The Relative Persistence of Bact. coli and Bact. Aerogenes in Nature, I. In Decaying Stumps, Bureau of Laboratories and Research, Department of Health, Chicago, 1931, http://thewatchers.us/EPA/2/1931-presistence-E-coli-Enterbactor.pdf

Total coliform Disease Table, http://thewatchers.us/deaths/disease/table-1-coliform.html

Tubiash, Haskell S., The Anaerogenic Effect of Nitrates and Nitrites on Gram-Negative Enteric Bacteria, Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md., AMERICAN JOURNAL OF PUBLIC HEALTH July, 1951, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1525593/pdf/amjphnation00425-0068.pdf

Walker, John., Bastian, Bob., Candidates for the Rest of the Story. 1994, http://deadlydeceit.com/EPA-PR.html

Walker, John, THE PROPERTIES OF SLUDGE / AKA BIOSOLIDS, R.B. Dean and J.E. Smith Jr., Proceedings of the Joint Conference on Recycling Municipal Sludges and Effluent On Land, July 9 - 13, 1973, page 8, http://thewatchers.us/science/properties-sludge-8.html

Washington State Health Department. http://www.doh.wa.gov/ehp/dw/programs/coliform.htm

WHO, Water Quality: Guidelines, Standards and Health, Chapter 13, Indicators of microbial water quality, 2001, http://www.who.int/water_sanitation_health/dwq/iwachap13.pdf

Wisconsin State Laboratory of Hygiene, Water Microbiology for State and Federal Agencies, 2010, http://www.slh.wisc.edu/ehd/watermicro/