Uncoiling E. coli: Detection and Treatment

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Introduction

Escherichia coli is a growing concern in today's society. Each year, millions of individuals over the world are infected, and tens of thousands die. Not only this, but substantial amounts of money are spent by individuals or the government in order to cover the costs of hospitals, creating a burden on both citizens and the economy. Shiga Toxin–Producing E. coli (STEC) in particular, is much more dangerous and deadly than other strains of the bacterium. The symptoms of the infection are often severe and life-threatening complications such as Hemolytic Uremic Syndrome (HUS) can arise. Despite this, there is a lack of treatment, detection and prevention of STEC, resulting in fatalities. This paper aims to look into aspects of STEC and summarize the respective findings. The first aspect provides background information on the topic, including in-depth statistics and research on HUS and the shiga toxin. The second aspect explores bacteria that are similar to STEC in order to investigate if treatments for the said bacteria could also be used for STEC. Finally, the last aspect of the topic outlines an experiment conducted to detect E. coli on store-bought lettuce.

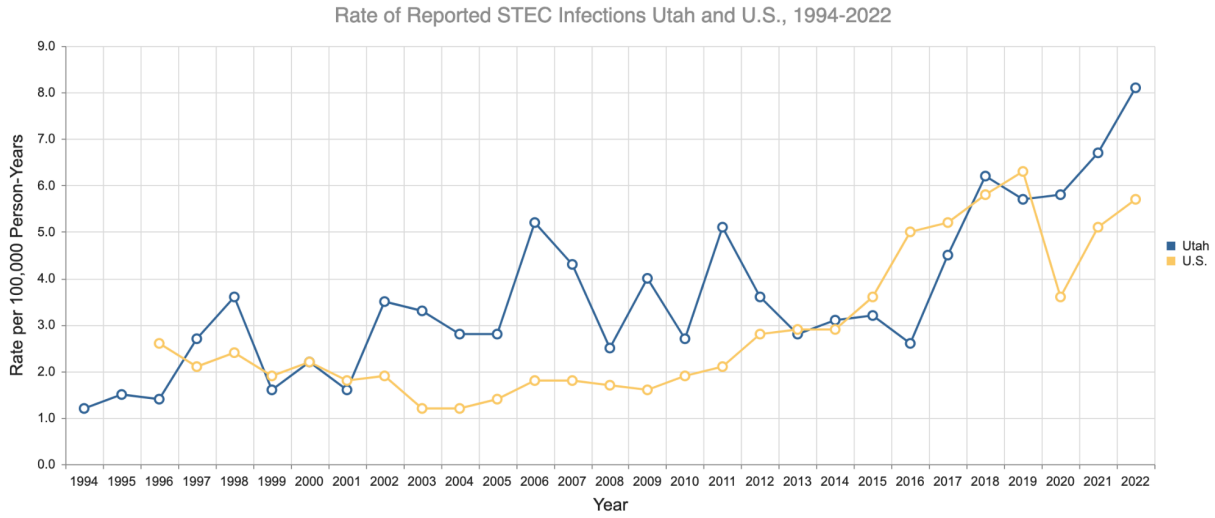
Background Information

While most strains of E. coli are harmless, STEC is a strain of the bacterium that belongs to the family of Enterohemorrhagic E. coli (EHEC) that tends to cause severe infection. Although E. coli is present in an individual's gut, those strains are relatively harmless, while STEC is not. STEC is associated with many cases of public outbreaks, such as the Calgary daycare case (Graveland, 2024), in which the outbreak stemmed from a contaminated meatloaf. Infection typically occurs through contact with contaminated food, most commonly ground beef, unpasteurized milk and fresh produce. E. coli is present in the intestines of cows, and when

slaughtered or during the processing stage, it can get onto meat consumed by humans. Similarly, E. coli on a cow's udder can contaminate milking equipment or raw milk which is not further pasteurized if not appropriately handled (*E. Coli*, 2022). Pasteurization is the method of removing pathogens in food or drink, such as milk, by heating said food in mild temperatures. Additionally, fresh produce can also become contaminated with E. coli through runoff from ranches or cattle farms. Certain types of produce are more susceptible to contamination, such as lettuce and spinach. Common symptoms of STEC include bloody diarrhea, stomach cramps and vomiting. Although anyone can contract the infection, typically children under 5, seniors over 65, frequent international travellers and individuals with weakened immune systems are more prone. Individuals with conditions including diabetes and cancer, have also been shown to have a higher risk of contracting STEC E. coli (Fatima, 2018). Infection rates are constantly increasing yearly, only showing a slight decrease in 2020 due to the COVID-19 pandemic as many individuals were quarantined within their homes and less likely to spread the infection. The Utah Department of Health & Human Services has said, "Effective prevention is the best treatment for STEC" (*IBIS-PH - Health Indicator Report - Foodborne Illness - Shiga Toxin-Producing "E. Coli" (STEC) Infections*, 2024).

Figure 1

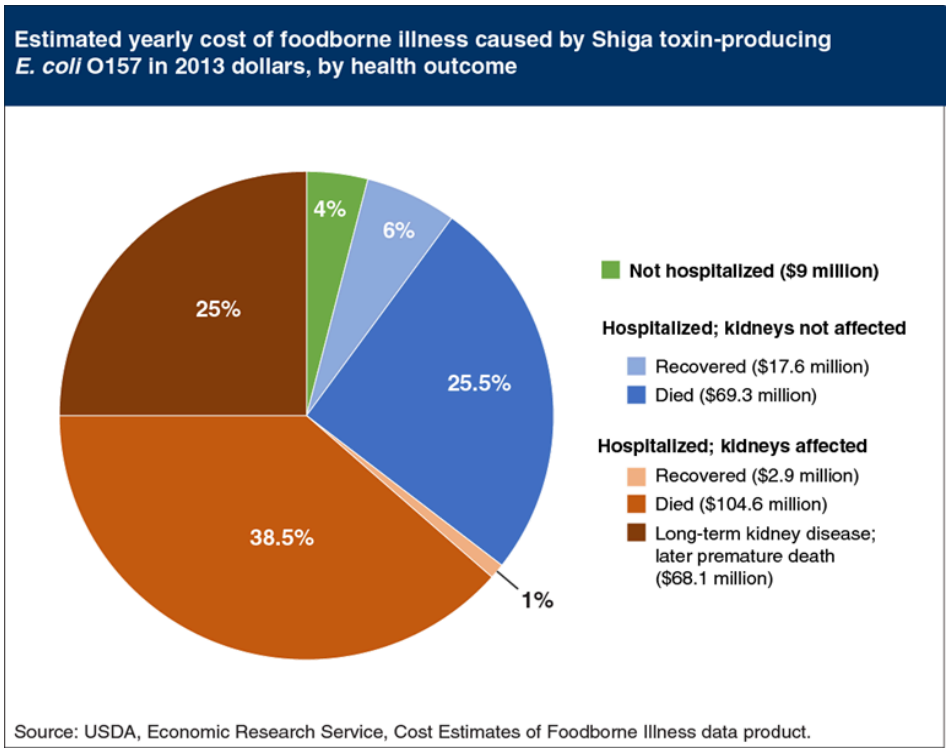
Rate of Reported STEC Infections in Utah and U.S., 1994-2022



Despite this, healthy adults typically take around a week to recover. On the other hand, vulnerable groups, particularly children under 5, can develop hemolytic uremic syndrome (HUS). HUS affects around 5%-15% of individuals with E. coli diarrhea (*Hemolytic Uremic Syndrome*, 2024). Symptoms are similar to those of STEC, including diarrhea, stomach pains, vomiting, headaches, fever and chills. HUS essentially blocks blood vessels in the kidneys, destroying blood cells and reducing platelet count, which are essential in preventing excessive bleeding. Although HUS predominantly affects the kidneys, it can also affect other organs, such as the heart, making it more dangerous. A study conducted by Gould et al. (2009) in 2002-2006 showed that out of 3634 cases of STEC, 218 (6.3%) developed HUS. 15.3% of all HUS cases were present in children under 5 due to their susceptibility from their developing immune systems. Overall, the fatality rate in those with HUS was 4.6% while in those without was comparatively less, at 0.6%. To a large extent, the substantial number of cases per year and the costs of expenses, such as hospital charges, place a heavy economic burden on both individuals and the government.

Figure 2

Estimated yearly cost of foodborne illnesses caused by Shiga-toxin producing E. coli 0157 in 2013 dollars, by health outcome



HUS occurs due to the production of the Shiga toxin, which is present in STEC. According to (*Shiga Toxin*, n.d.), the Shiga toxin binds to receptors through a molecule that attaches to another (called a binding-moiety). From this point onwards, it is carried to two organelles responsible for protein production and transportation, the Golgi apparatus and the endoplasmic reticulum. After this, the Shiga toxin essentially "deploys" its toxicity. Fundamentally, STEC releases the Shiga toxin which damages cells in the intestinal lining of the stomach, causing a damaging, toxic effect to the body. There are also various groups of the Shiga toxin, including Stx1 and Stx2. Typically, Stx2 is considered the more potent and toxic of the two. Different E. coli strains can present with either Stx1 and Stx2 or both. For example, a study conducted by Tahamtan et al.

(2010) in 2007-2008 found that in a total of 146 strains, both Stx1 and Stx2 genes were found in 51 strains, approximately 35%.

The Shiga toxin inhibits protein synthesis, a process that cells use to create proteins, an essential part of cell survival. Protein synthesis occurs in the ribosomes in the Rough Endoplasmic Reticulum, which is responsible for producing proteins for the rest of the cell to function. Shiga toxin targets a part of the ribosome called 60S subunit, which catalyzes peptide formation, accelerating the process of two amino acids joining together to form a protein. Specifically, the toxin removes an adenine from a structural ribosomal RNA called 28S RNA, starting the process of apoptosis. Apoptosis is a type of cell death in which a series of molecular steps are performed, which lead to the eventual death of the cell. Outside of the scope of E. coli, apoptosis is often used by the body in stages of development and in ensuring that damaged body cells are rid of to prevent them from causing further harm.

The Shiga toxin has two subunits, A and B. The A subunit acts as a “scissor”, removing an adenine from 28S RNA in order to inhibit ribosome function. The A subunit also has fragments, A1 and A2, linked by a strong, covalent bond called a disulfide bond. If the fragments separate in the cell of the toxin, then, it causes cell damage as the A1 fragment, which contains the toxins of the cell, separates from the A2 fragment. Depending on the variant of the toxin, they can also be activated by the mucus in the intestinal lining. This mucus can separate the A1 and A2 fragments, which again, causes the toxic activity of the cell. In addition, the activation of the toxin also varies based on the bond between the A and B subunits. The B subunit aids the toxin in binding to the cell. Shiga toxins can be phage-encoded or chromosomally encoded. Phage-encoded toxins, including Stx1 and Stx2, have the genes of the toxin carried by bacteriophages, which are viruses that infect bacteria. In other variants of the toxin, these genes

are encoded in the bacteria's DNA. Shiga toxin requires a receptor to infect a cell. These receptors are molecules called globotriaosylceramide (Gb3). Essentially, the Shiga toxin binds to a cell using the Gb3 pathway, except for Stx2e, which instead uses Gb4, also known as globotetraosylceramide (*Shiga Toxin*, n.d.). Based on the amount of receptors present in the cell, the toxin is more or less likely to bind to it. The more receptors in the cell, the more susceptible it is to the toxin and vice versa. The bacteriophages infect the healthy E. coli in the gut and the infected E. coli releases a substance called the Shiga toxin. STEC E. coli produces the Shiga toxin but the toxin affects the bacteria that makes the toxin itself. This is called "bacterial altruism". The bacteria essentially make a "sacrifice" to continue the production of the toxin as only a portion of the population is affected while the rest are not. This works towards a "greater good" as although a few bacteria die during the process, the rest are able to survive and continue exerting the toxic effect on the other cells (Łoś et al., 2013).

Treatments

Despite the harmful effects of STEC, there is currently a lack of treatment options. Presently, the treatment of the illness relies on supportive therapy, including rehydration therapy or kidney dialysis in severe cases (Mühlen & Dersch, 2020). Rehydration therapy treats the dehydration caused by diarrhea from the infection while dialysis cleans blood and removes excess fluids when kidneys are unable to function due to HUS. However, these treatments can be time-consuming and expensive, which many individuals and the government may not be able to afford over long periods of time. In particular, antibiotics are not recommended for STEC. A reason for this include that antibiotics can increase the risk of developing HUS due to increased shiga toxin production. Another reason is that in some cases, bacterial SOS response can be

initiated, particularly with broad-spectrum antibiotics including ciprofloxacin, which can eventually lead to mutations in the DNA of the bacteria, causing resistance towards the antibiotic (Mühlen & Dersch, 2020). Due to the lack of treatments, we looked into similar bacteria to E. coli and studied their treatments to observe if any could be used for STEC. One of the most closely related bacteria to E. coli is Salmonella. Both bacteria are fundamentally similar as Salmonella evolved from E. coli millions of years ago (Baker, 2018).

Salmonella is a bacterial disease that affects the gastrointestinal tract. The most likely sources of infection are contaminated food or water, especially in countries without access to clean drinking water or proper sewage disposal. Symptoms include diarrhea, fever, nausea, vomiting, chills, headache and stomach cramps. Like STEC, healthy individuals typically recover within a few days to a week (*Salmonella Infection*, 2022). However, potentially fatal complications can develop if the infection spreads further. According to (Lavigne & Blanc-Potard, 2008), both E. coli and salmonella have evolved to become pathogenic through gaining virulence "tools" that can cause disease to humans. Such tools include adhesions to stick to human cells, type III secretion systems, a bacterial secretion system used commonly by Gram-negative bacteria, like E. coli, toxins and the ability to remove iron from host cells. These are typically acquired through horizontal gene transfer, which is the movement of genes or genetic material between organisms.

The gene transfer is driven either through pathogenicity islands, plasmids, or prophages. Pathogenicity islands are genetic elements that microorganisms obtain through horizontal gene transfer. In non-pathogenic strains of bacteria, these islands are not typically present. Plasmids are circular DNA in bacterial cells while prophages are viruses that infect bacteria. These factors are essential to study as they can be used to diagnose the type of infection and to develop

treatments. Additionally, understanding bacterial evolution regarding virulence is crucial in the face of increasing antibiotic resistance. Currently, there are a few options for the treatment of salmonella.

Firstly, antibiotics are a viable treatment method. However, as previously discussed, antibiotics cannot be used for STEC as they can increase the risk of HUS and have not been shown to have any particular remedial effect. An example of an antibiotic used for salmonella is ciprofloxacin (Owens, 2024), which is a part of the quinolone family, a class of broad-spectrum antibiotics. A study conducted found that, around 73% of E. coli isolates, a culture of microorganisms used for research purposes, were found to be resistant to ciprofloxacin (Mandal et al., 2012). Additionally, the quinolone family has also been shown to initiate bacterial SOS, which can lead to bacteria being able to mutate their genome, eventually contributing to antibiotic resistance. Hence, antibiotics are not usable in the case of STEC E. coli.

Secondly, studies have shown that probiotics can help prevent STEC or reduce the severity of the infection. Probiotics are live bacteria that benefit the body, particularly the digestive system, and keep the gut healthy. A few examples of probiotics used for salmonella include lactobacilli and bifidobacterium. According to (Lee et al., 2021), lactobacillus inhibits STEC proliferation, which is a rapid increase in numbers, as it produces hydrogen peroxide, lactic acid, IgA and leukocyte activity. Bifidobacterium inhibits the production of Stx, compared to the growth, by increasing the pH of the stomach and increasing the concentration of acetic acid (Asahara et al., 2004). In conclusion, the lack of treatments other than supportive therapy and the ineffectiveness of antibiotics is a growing concern. However, various studies have shown that probiotics, including lactobacillus and bifidobacterium, which are also treatments for salmonella, are potentially usable for the STEC E. coli.

Detection

Purpose & Applications

The purpose of this experiment was to run a PCR test on store-bought lettuce to detect the presence of E. coli. Though there are currently detection methods for STEC E. coli, areas of improvement are present. These methods may be time-consuming or expensive, again adding to the economic and financial burden on individuals. By conducting this experiment, future work can include creating a faster, effective version of PCR for the detection of STEC. This method could then be used on a wider-scale, such as protecting public health and safety by ensuring consumed food is not contaminated.

Research

PCR, or polymerase chain reaction, is a method in which segments of DNA rapidly divide, providing scientists with the opportunity to study or detect the DNA section. This method is often used for the detection of infectious diseases, including STEC E. coli. One of the fundamental concepts in PCR is primers. Primers are short sections of single-stranded DNA which dictate the segment of the DNA that is to be copied. Synthetic primers are also known as oligonucleotides. Primers also start DNA synthesis, which is the process in which a new DNA strand is created from a template. As the DNA segment divides, it results in millions or billions of copies of the segment in a short period of time, allowing scientists to analyze information provided by the test.

Procedure

1. **Grow the E. coli:**
    - Materials: E. coli stock cultures, agar plates, applicator sticks, incubator
    - Process (~1 day):
        - **i.** Remove chunk of bacteria from stock culture vial and streak onto LB agar plate using applicator stick
        - **ii.** Incubate overnight at 37℃
2. **Obtain Lettuce:**
    - Type of lettuce: Romaine lettuce (more susceptible to infection)
    - Amount: 2 heads of lettuce
    - Parts: Younger leaves near the top of the lettuce (more exposed) and leaves near the crown
        - i. Use other parts (eg. outer leaves, stem) to increase likelihood of detection
3. **Sample Preparation**:
    - Homogenize:
        - i. Material: Blender (disinfect afterwards)
        - ii. Add sterile buffer in order to help aid homogenization (essentially making it one consistent mixture)
        - iii. Centrifuge after homogenization to separate excess liquid
    - Washing: May reduce the concentration of bacteria on the lettuce, hence, not washing the lettuce may be easier to work with
    - Filtration:
        - i. Remove large pieces to make process easier
4. **Additional Procedure**:

- Colony PCR:
    i. Preform a test using colony PCR
    ii. Small amount of bacterial cells from a colony are used as a template for PCR and then added to the “Master Mix” and then PCR is preformed (essentially confirms is bacteria is E. coli or not)

5. **PCR**:
    - Select target gene for E. coli (ybbW)
        i. Design primers to flank target gene (amplify the DNA segment -> initiates DNA synthesis)
        ii. How much to use: 2.5 µl of forward primer and 2.5 µl of reverse primer in PCR tubes of 200 mL (based on protocol stated below)
    - Master Mix Protocol (*Protocol for Q5® High-Fidelity 2X Master Mix*, n.d.):
        i. “For 50µl reaction, following components and quantities are required
            1. Master Mix - 25µl
            2. 10 µM Forward Primer - 2.5µl
            3. 10 µM Reverse Primer - 2.5µl
            4. Template DNA
            5. Nuclease Free Water - up to 50µl”
    - Add lettuce sample into PCR tubes containing Master Mix
6. **Detection & Analysis:**
    - Gel electrophoresis:
        i. Gel preparation
            1. Prepare gel buffer and agarose powder

2. Add powder to gel buffer and heat until dissolved
3. Allow the solution to cool and then add a DNA staining dye
4. Pour solution into tray and let gel solidify
5. Remove comb to create room for samples

ii. Sample

1. Take volume of PCR product and add a loading buffer and mix
2. Add DNA ladder into open space

iii. Electrophoresis

1. Fill chamber with gel buffer and agarose gel
2. Put samples and DNA ladder into open room
3. Run gel electrophoresis

7. **Interpret Results**
    - Compare bands to DNA ladder
8. **Results**
    - Will be presented at CYSF

Sources of error

Potential sources of error may include inadvertent contamination with bacteria other than E. coli. Additionally, the filtration and homogenization process could interfere with the PCR process, contributing to a possible error in the process.

Conclusion

STEC cases are increasing, and despite this, there is still a lack of effective treatment and detection methods. The findings of this paper show that potential treatment options include probiotics, such as lactobacillus and bifidobacterium, that can act in place of antibiotics without increasing the risk of HUS. Further investigation on this topic can help reduce STEC E. coli outbreaks and related issues, such as economic burdens. Additionally, aiming to develop effective and efficient detection methods is an area of future research.

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