Beware: What's Growing on Your Produce? Pathogenic Contaminants?

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Abstract

For the last few decades foodborne poisoning has been on the rise due to an increase in the consumption of fresh fruit and vegetables. The contamination of produce and food in general can lead to the rise of health issues in consumers. Some bacterial causes of food-related illnesses are *Escherichia coli*, *Listeria monocytogenes*, and *Enterobacter cloacae*. These bacteria can cause diarrhea, vomiting, nausea and abdominal pain. Typically, viruses do not grow on contaminated vegetables and fruit but can survive long enough to cause life threatening illnesses in humans. According to the Centers for Disease Control and Prevention (CDC), there were multiple *Escherichia coli* O157:H7 outbreaks across the United States and Canada. The source of this Shiga- toxin producing *Escherichia coli* strain was leafy greens such as romaine lettuce. Produce contamination can occur during the planting stage. If produce is planted on fields where flooding has occurred or where animals once grazed, microbial contaminations may arise. Contaminated water can also be a source of contamination. From water used during the planting stage to water used when washing off produce, contamination may arise. The purpose of this study is to determine the prevalence of contamination of fresh produce in from local, domestic, and international origins. In this study, six samples were gathered from local, domestic, and international origins. The samples purchased from local stores were raspberries, strawberries (Louisiana), carrots, lettuce (California), cherry tomatoes, blackberries (Mexico) and grapes (Chile). All samples were initially cultured in Nutrient and Tryptic Soy broth . Then those were subcultured on nutrient agar plates to isolate colonies. The following selective agar media were used: Hektoen Enteric, MacConkey, and Eosin Methylene Blue. These media are used to identify and differentiate coliforms. The resulting bacteria grown were coliforms such as Escherichia coli, Salmonella, and Shigella.The ability of pathogens to attach to the surface of the produce and get inside put constraints on the control of the contaminants. There is a need for better ways for preventing contamination at the farm, while packing, processing, transporting, and preparation in the kitchen. Future considerations include more sampling, and DNA and RNA analysis.

Introduction

- According to the Center for Science in the Public Interest, fresh produce is the cause of most foodborne illnesses in the United States. (Fresh Produce Remains Leading Cause of Outbreaks. Dec. 2018)
- Produce can be contaminated with harmful bacteria that could lead to consumer illnesses. (The Scientific World Journal. May 2014).
- According to the CDC, there were multiple Escherichia coli O157:H7 outbreaks across the United States and Canada. (Multistate Outbreak of E. coli O157:H7 Infections. Jan. 2018)
  - The source of this Shiga- toxin producing Escherichia coli strain was leafy greens such as romaine lettuce.

Introduction

- Some bacterial causes of food-related illnesses are *Escherichia coli, Salmonella enteritidis, Listeria monocytogenes, and Enterobacter cloacae.*
- CDC estimates that each year 48 million people get sick from a foodborne illness, 128,000 are hospitalized, and 3,000 die. (Foodborne Illnesses and Germs. Feb, 2018)
- Over a third of the country's vegetables and two-thirds of the country's fruits and nuts are grown in California. (California Department of Food and Agriculture. Feb, 2016)
    - Grapes, lettuce, strawberries, and tomatoes were in California's top-10 valued commodities for the 2016 crop year

Introduction

- On January 27, 2016, Dole voluntarily recalled all salad mixes produced in the Springfield, Ohio processing facility because of a multistate outbreak of Listeria monocytogenes infections (listeriosis).
  - 19 people were affected; all hospitalized.
  - 9 different states; 1 death.
- Hazard Analysis Critical Control Point (HACCP)
  - An internationally recognized system for reducing the risk of safety hazards in food.
  - Requires that potential hazards are identified and controlled at specific points in the process. Including biological, chemical or physical hazards.
  - Any company involved in the manufacturing, processing or handling of food products can use HACCP to minimize or eliminate food safety hazards in their product.

Purpose

- The purpose of this study is to determine the prevalence of contamination of fresh produce from local, domestic, and international origins.

Methodology

- First, samples were collected from local supermarkets.
- The samples collected were
  - Strawberries (Louisiana)
  - Raspberries (Louisiana)
  - Grapes (Chile)
  - Lettuce (California)
  - Carrots (California)
  - Tomatoes (Mexico)
  - Blackberries (Mexico)

Methodology

- Initially, the samples were cultured in Nutrient and Tryptic Soy broth.
  - General purpose media
- Then subcultured on nutrient agar plates.
  - To isolate colonies
- Each sample was further subcultured onto selective medias:
  - Eosin Methylene Blue Agar (EMB),
  - Hektoen Enteric Agar (HE)
  - MacConkey Agar

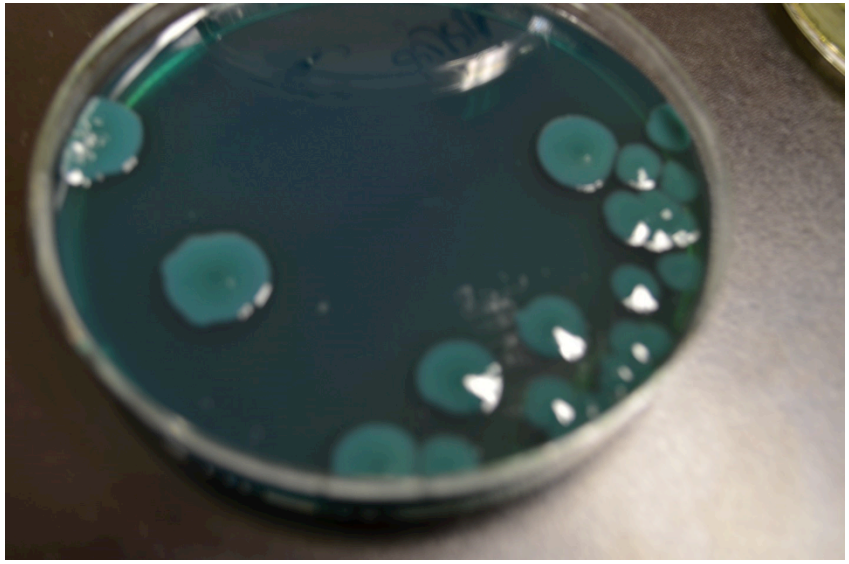
Figure 1: Samples placed in broth

Results: Table 1

Selective Media	**Samples**	**Results**	**Bacteria Identification**
Hektoen Enteric (HE)	Tomatoes Strawberries, lettuce	Salmon-orange colonies Greenish-blue colonies	*Enterobacter aerogenes* *Salmonella enteritidis* *Shigella flexneri*
Eosin Methylene Blue (EMB)	Blackberries, grapes, tomatoes, carrots Strawberries	Green metallic sheen colonies Pink colonies	*Escherichia coli (E. coli)*, *Klebsiella pneumoniae* *Enterobacter aerogenes*
Levine EMB	Carrots, Raspberries	Pink colonies	*Enterobacter aerogenes*
MacConkey	Tomatoes	Pink colonies	*Enterobacter aerogenes* *Escherichia coli*

Results: Hektoen Enteric Agar

Figure 2: Louisiana Strawberries*, California lettuce-green-blue moist raised colonies-*Shigella flexneri*, *Salmonella enteritidis*



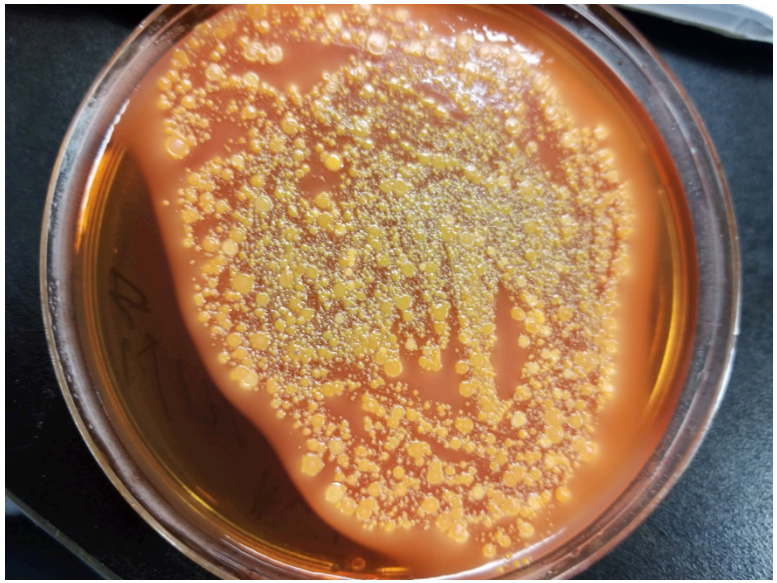


Figure 3: Mexico tomatoes-orange colonies-*Enterobacter aerogenes*

Results: Eosin Methylene Blue

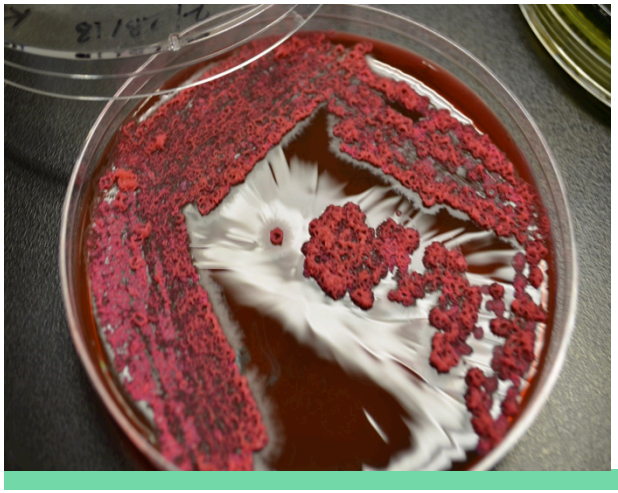


Figure 4: Louisiana Strawberries, -pink rough colonies – *Enterobacter aerogenes*.

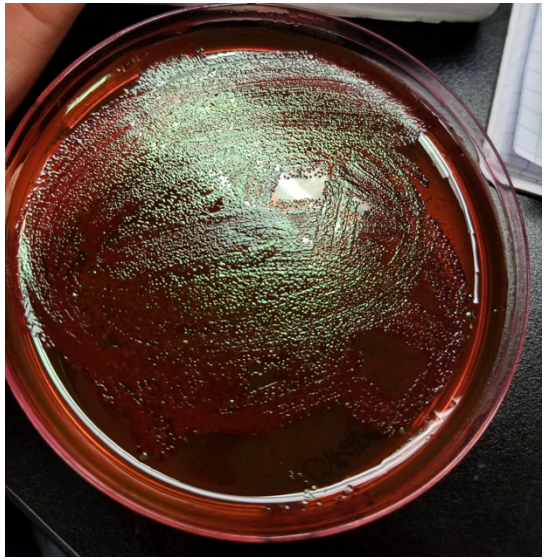


Figure 5: Chile Grapes- green metallic sheen- *Klebsiella pneumoniae, E. coli*.

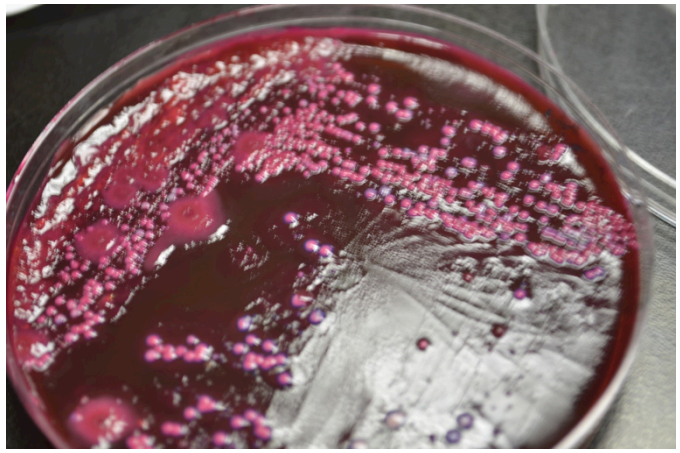


Figure 6: Levine EMB-California carrots*, Louisiana Raspberries-pink colonies-*Enterobacter aerogenes*

Results: MacConkey Agar

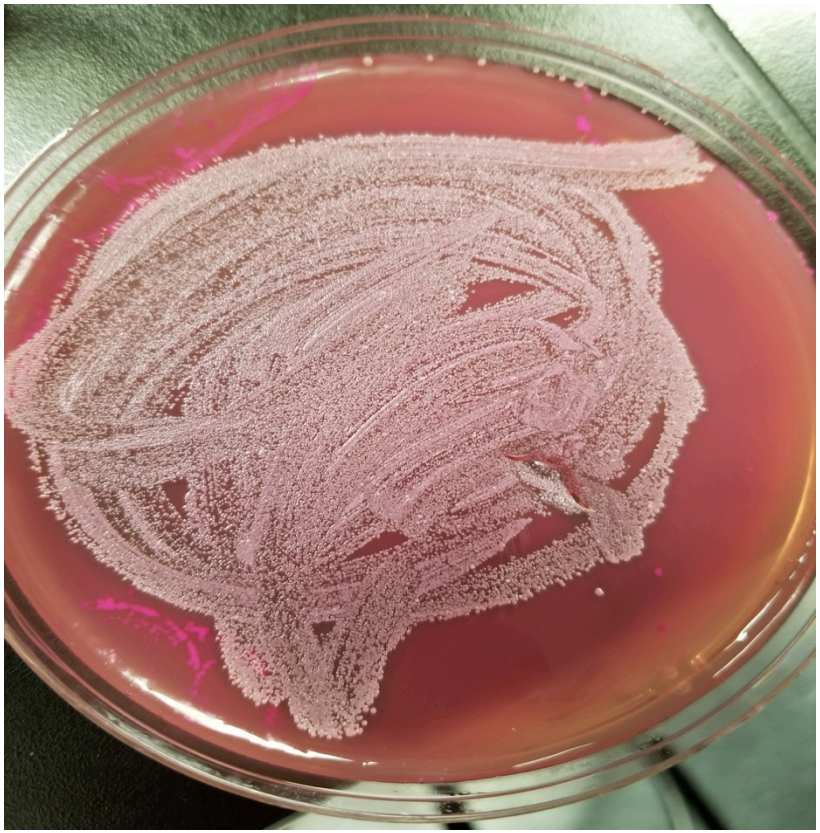


Figure 7:
Mexico Cherry tomatoes-
pink colonies-
Enterobacter aerogenes,
Escherichia coli

Discussion

- Hektoen Enteric Agar
  - Selective medium
  - Differentiates *Salmonella* and *Shigella* species from other Gram-negative enterics.
  - *Enterobacter aerogenes*
  - *Salmonella enteritidis*
    - The leading species of *Salmonella* that causes illness in the United States.
    - Causes fever, abdominal cramps, and diarrhea beginning 12 to 72 hours after consuming a contaminated food.
    - Usually lasts 4 to 7 days, and most persons recover without antibiotic treatment.
  - *Shigella flexneri*
    - Can cause serious gastrointestinal infections; diarrhea
    - Causes about 500,000 cases of diarrhea in the United States annually
    - Sources are contaminated food or water
    - Salads are most often associated with Shigella outbreaks because they involve a lot of hand contact in their preparation, and raw vegetables contaminated in the field.

Discussion

- Eosin Methylene Blue
  - selective for Gram-negative organisms
  - Commonly used to test for the presence of coliforms
    - Coliforms suggest the presence of fecal contamination and the presence of enteric bacteria which is found in the colon.
  - *Klebsiella pneumoniae*
    - Causes pneumonia, bloodstream infections, wound or surgical site infections, UTI and meningitis
    - Normal flora of human intestines
  - *Enterobacter aerogenes*
  - *Escherichia coli*

Discussion

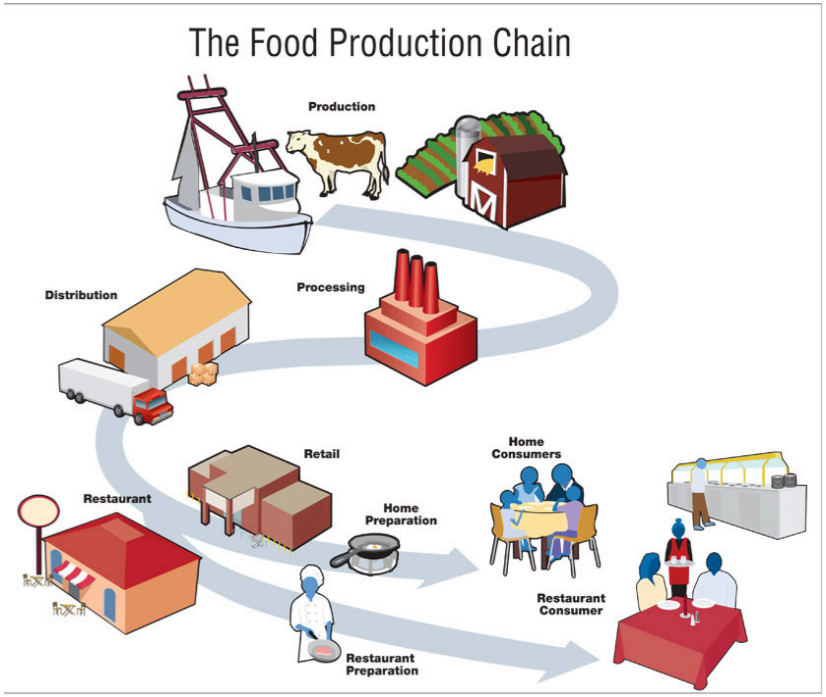
- MacConkey
  - Selective media
  - Used to isolate and differentiate members of the *Enterobacteriaceae* family.
  - *Enterobacter aerogenes*
    - Ubiquitous bacteria in the environment; found naturally in soil, fresh water, vegetables, and human and animal feces.
    - Opportunistic pathogen
    - Cause lower- respiratory infections, urinary tract infections, and infections of the skin.
  - *Escherichia coli*
    - Found in the environment, foods, and intestines of people and animals.
    - Some kinds can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.

Cleaning Fresh Produce

- Fit Organic Fruit & Vegetables Wash is a product that can be used.
- Rinsing is an effective method for washing fruits and vegetables.
- Washes and treatments can be brought from the grocery store.
- The washes reduce pesticides and remove bacteria, but do not completely eliminate them.

Conclusion

- The samples from Louisiana, California, Mexico and Chile were proven to be contaminated with *E. coli, Enterobacter aerogenes, Shigella flexneri, Salmonella enteritidis,* and *Klebsiella pneumoniae.*
- Proper handling of fresh produce should be used from the first stages of planting until it reaches the dinner table.
- Contamination can occur at any point along the chain, during production, processing, distribution, or preparation in the kitchen.



Conclusion

- Contaminated water, cross contamination of feces from domestic and wild animals, the grazing of animals in the fields, and unsanitized equipment or transport vehicles are all considered to be sources.
- Vehicles transporting finished products should be sanitized, properly loaded to provide adequate air circulation, and maintained at proper temperatures.
- Finally, consumers should be informed how to properly handle produce, particularly in the case of new generation products such as modified atmosphere packaged produce (MAP's).
- Foodborne outbreaks from contaminated fresh produce have been increasingly recognized in many parts of the world.
    - This reflects the increasing consumption of fresh produce, changes in production and distribution, and a growing awareness of the problem on the part of public health officials.

Future Considerations

- Additional sampling of products
- Different test to differentiate these pathogenic organisms
  - DNA and RNA analysis.
- Differential and/or biochemical tests to differentiate the organisms based on biochemical characteristics.
  - Lactose Broth with phenol red
  - Mannitol Broth with phenol red

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Questions?