



# **The Challenge of Emerging Foodborne Pathogens: Non-O157:H7 STEC *E. coli* and *Cronobacter sakazakii***

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# What is an Emerging Pathogen?

- A phrase to describe a pathogen that took a long time to be recognized?
  - *E. coli* O157:H7 is likely evolved more than 100,000 years ago
  - *Cronobacter* was first reported as contaminating milk powder and causing illness in infants in the mid-1960s
  - There may not be very many truly “new” pathogens

# Emerging Foodborne Pathogens of this Generation

- *E. coli* O157:H7 and non-O157 variants
- *Cronobacter* (formerly *Enterobacter*) *sakazakii*
- *Listeria monocytogenes*
- Norovirus
- *Yersinia enterocolitica*
- *Campylobacter jejuni*
- BSE prions



# Why Do We Fail to Identify New Foodborne Pathogens?

## *Cronobacter sakazakii*

- A pathogen that causes illness infrequently and that most notably attacks young infants
- Taxonomically, it is found with bacteria widely thought to be harmless commensals
- It is often isolated from adult clinical specimens where it does not cause illness.

# Why Do We Fail to Identify New Foodborne Pathogens?

## Non-O157 STEC

- Difficult to isolate and to uniquely identify
- Taxonomically and serologically similar to non-pathogenic *E. coli*
- Difficult to distinguish pathogenic from non-pathogenic types of *E. coli*
- Don't fully understand *E. coli* virulence
- More than just the "Big 6" serotypes (O26, O111, O103, O121, O45 and O145)

# Attributes that Conceal a Foodborne Pathogen

- Attacks only a subset of the population
- Cases are uncommon or even rare
- A virulent variant of a species that includes mostly avirulent types
- A lack of distinguishing phenotypic characteristics
- Commonly occurs in foods and the environment

## ***Cronobacter sakazakii***

- Causes the most severe infections in young infants, typically < 6 months of age
- Opportunistic pathogen in older hosts, usually found in normally sterile body sites
- Septicemia or meningitis in infants
- FDA learns of fewer than 10 cases a year on average, usually isolated cases
- All *C. sakazakii* are presumed virulent, multiple serotypes cause illness

## Non-O157 STEC

- Many strains of *E. coli* produce Shiga toxin, but do not cause illness – over 600 serotypes of STEC have been identified
- Virulent and avirulent strains occur in the same serotype
- Multiple virulence genes
- [http://ecdc.europa.eu/en/publications/Publications/1106\\_TER\\_EColi\\_joint\\_EFSA.pdf](http://ecdc.europa.eu/en/publications/Publications/1106_TER_EColi_joint_EFSA.pdf) - an excellent summary of *E. coli* virulence and the recent O104:H4 strain

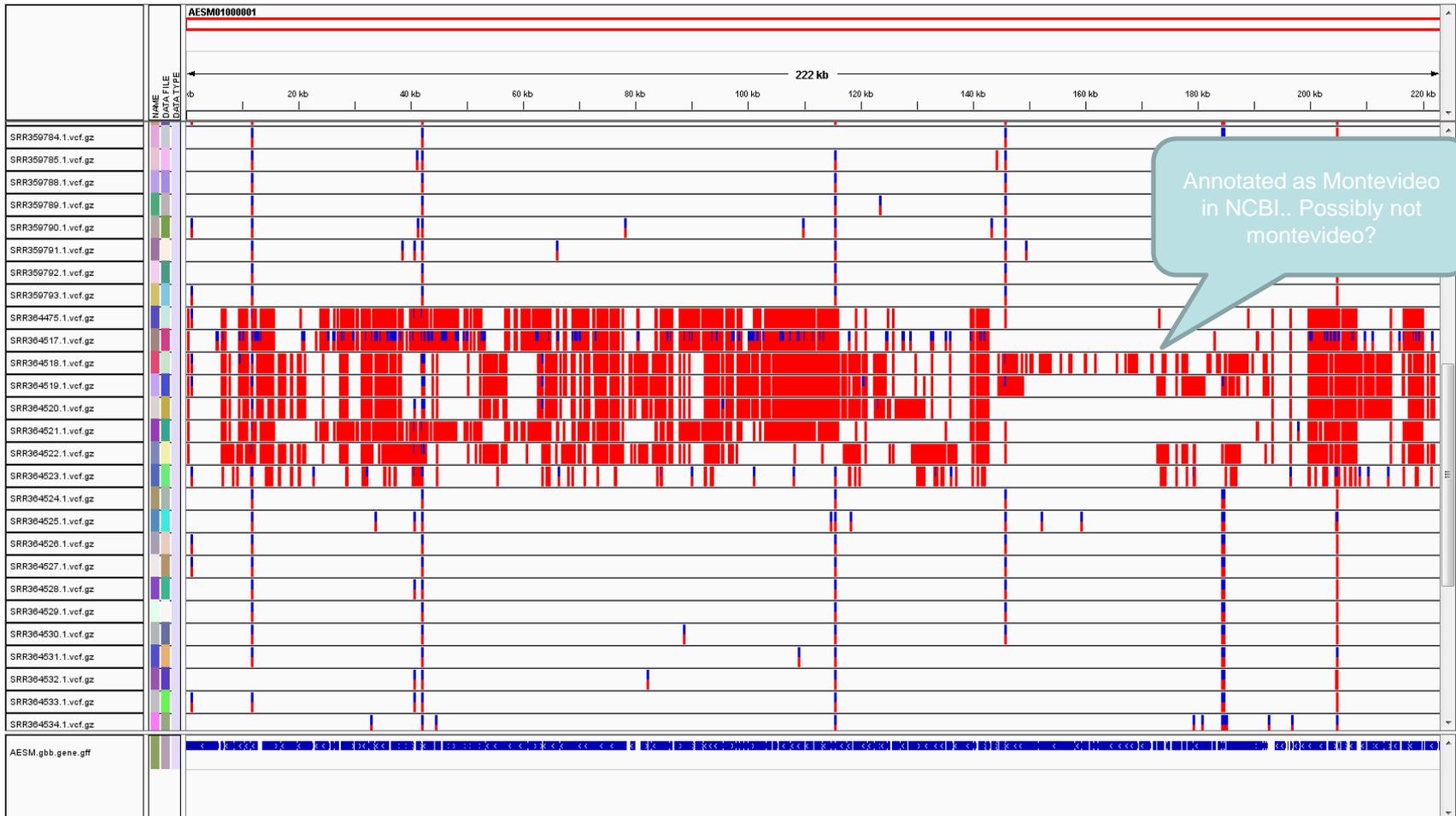
## ***E. coli* O104:H4**

- Newest non-O157 STEC to emerge
- Unique characteristics:
  - Sorbitol fermenting
  - Serotype: O104:H4
  - Shiga toxin 1: - (negative)
  - Shiga toxin 2 (subtype 2a): + (positive)
  - Intimin (eae): - (negative)
  - Enterohemolysin: - (negative)
  - Enteroaggregative *E. coli* virulence plasmid:
    - aatA: + (positive) aggR: + (positive) aap: + (positive) aggA: + (positive) aggC: + (positive)

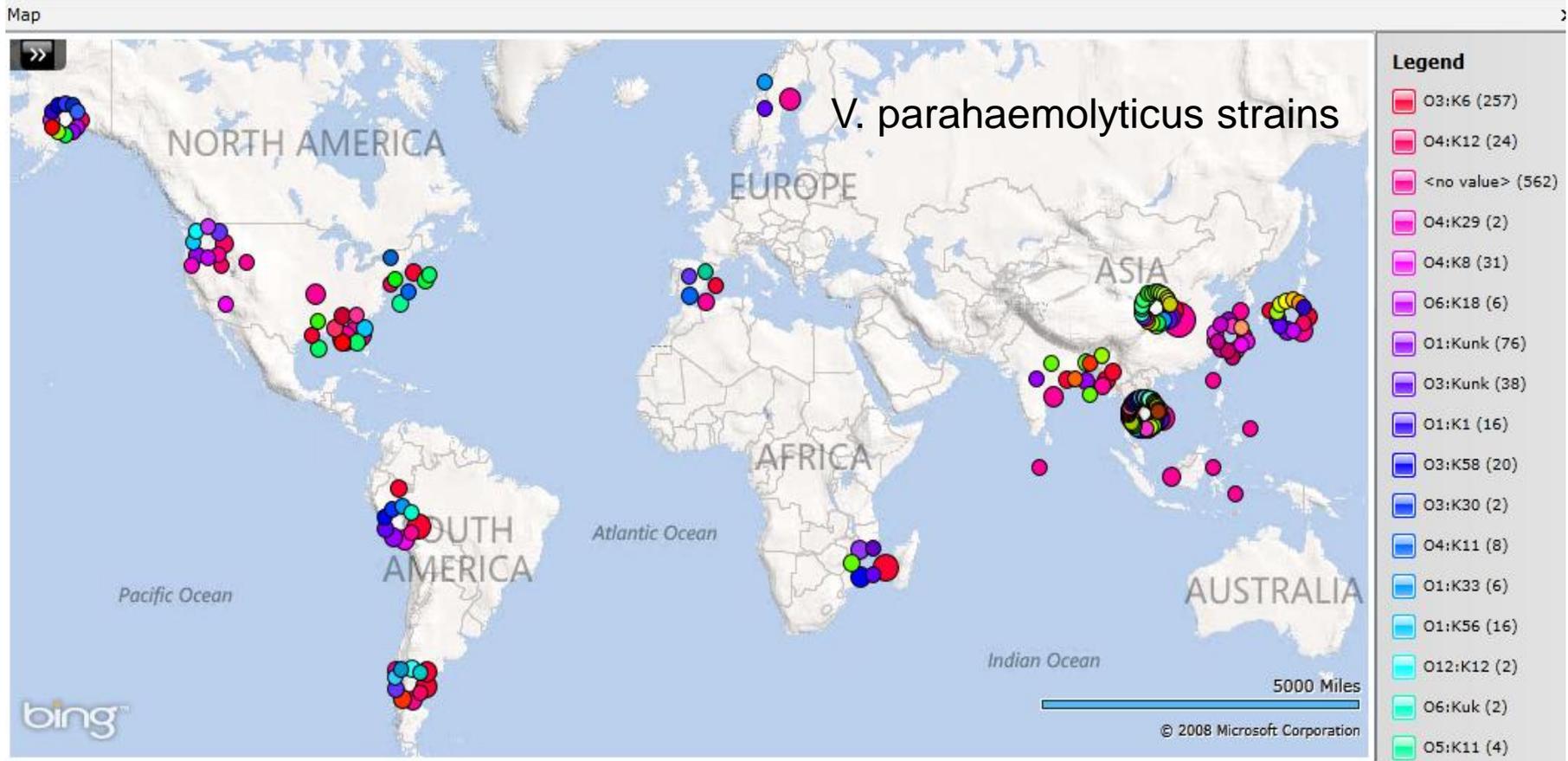
# What Can We Do to Detect Emerging Pathogens?

- We need a global system for fully characterizing outbreak strains and sharing that data in near real-time
  - Has this strain caused illness before
  - Are there characteristics of this strain that cause us to think it may be virulent
- Two technologies may help:
  - PATRN – an open source database
  - Next Generation Sequencing

# PATRN - Open-Source Data Visualization Tools

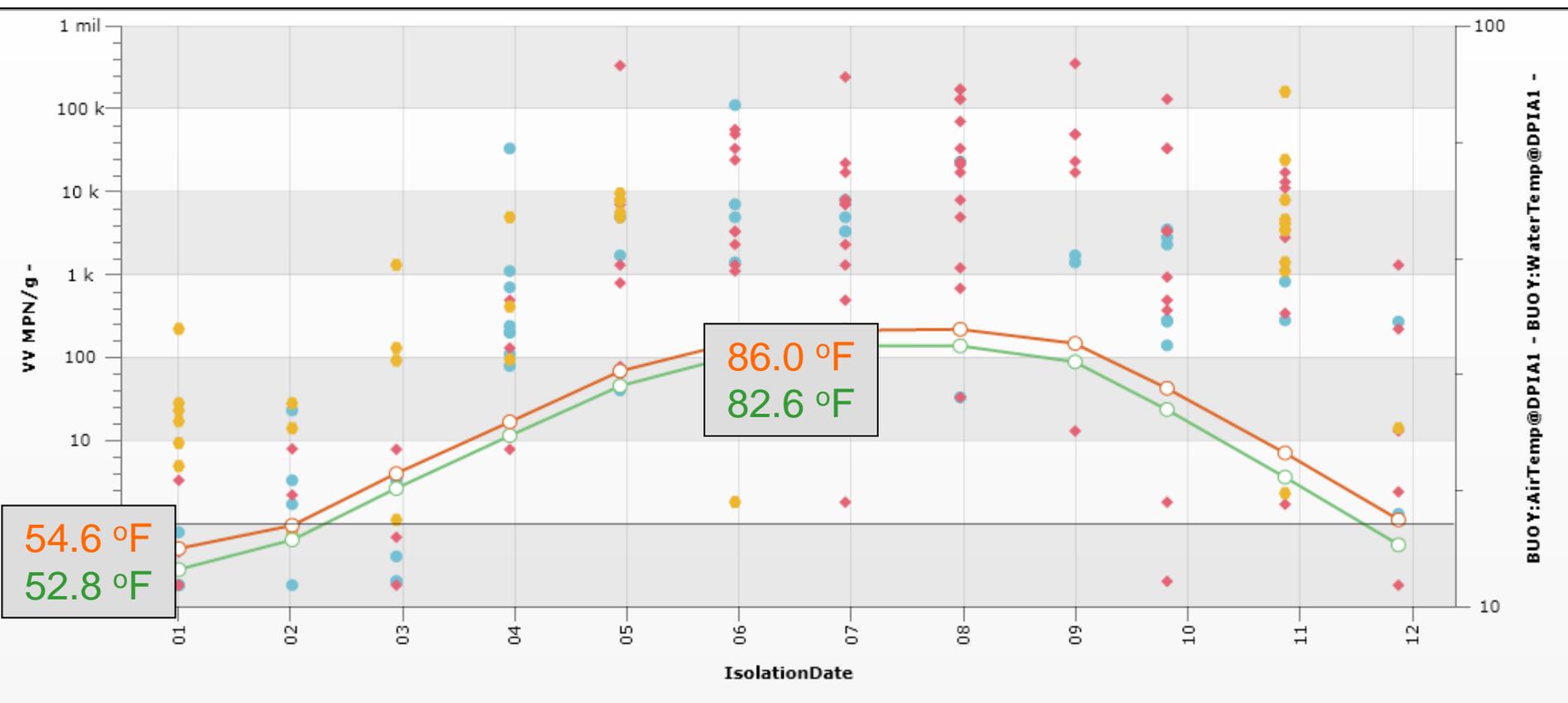


# PATRN – Geographic Display Strain Source





# PATR – CFSAN Laboratory and NOAA Buoy Data



● Florida    ◆ Louisiana    ● Texas    — Air Temp    — Water Temp

## Next-Generation sequencing

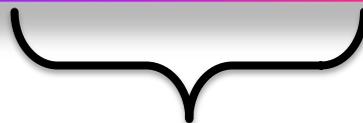
# Next-Generation sequencing can be used to address different facets of outbreak response:

- Have we seen this isolate before? (Compare to reference isolates)
- Do these clinical isolates form a cluster (i.e. is it outbreak or background)? (Compare to reference and other outbreak isolates)
- Is there a link between food/environmental and clinical isolates? (Compare to reference, clinical, and food/environmental isolates)

**Same PFGE Pattern**  
(but we suspect that not everything is related)

This region contains isolates where we are comfortable that PFGE will differentiate unrelated isolates

**Different PFGE Patterns**  
(but we suspect they may be related)

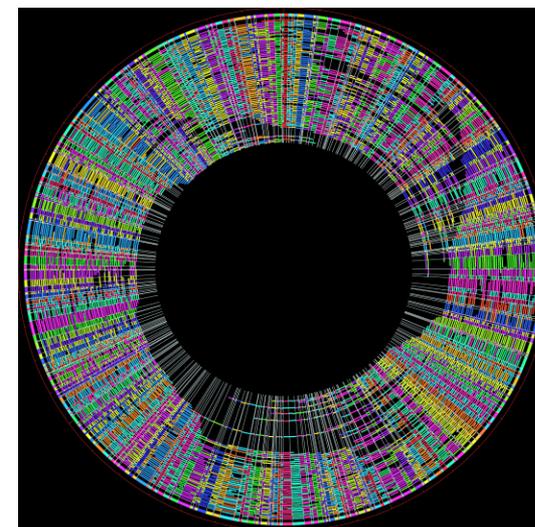
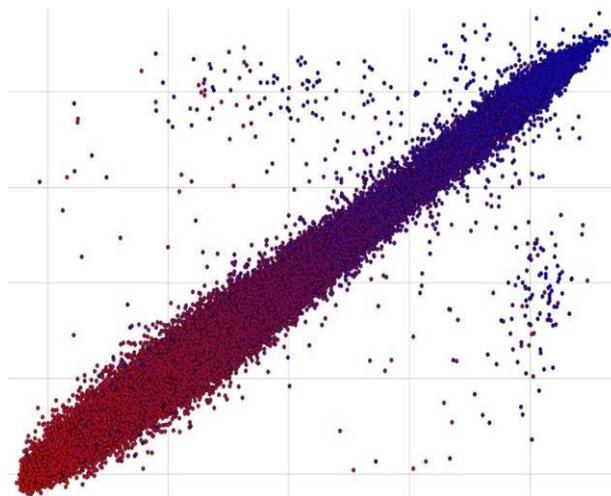
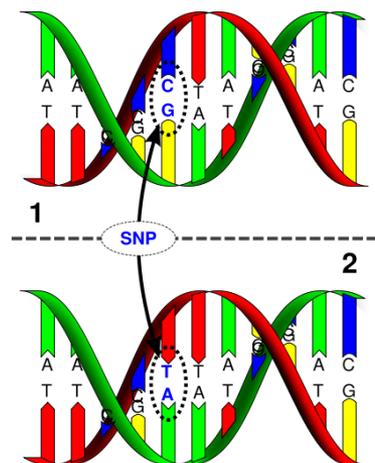


# Microarray and other next-generation genomic applications

SNPs

Gene content

Maps



- **Applied research on a genomic continuum:**
  - **Single Nucleotide Polymorphism (SNP) Panels**
  - **Custom Microarray Whole Genome Genotyping**
  - **Optical Map Gazing for Unique Chromosome Architecture**