Microorganisms Causing Foodborne Disease

Family Enterobacteriaceae

Shigella and Yersinia enterocolitica

Shigella

- 4 serogroups and species
  - *S. dysenteriae* (serogroup A)
  - *S. flexnerii* (serogroup B)
  - *S. boydii* (serogroup C)
  - *S. sonnei* (serogroup D)

Shigella

- Characteristics of the microorganism
  - Non-motile
  - Does not ferment lactose
  - Gas is not produced from glucose fermentation
  - IMViC is +++-
  - Does not produce lysine decarboxylase
  - *H₂S* and urease negative
Shigella

- Similar resistance and growth patterns to those of other enterobacteriaceae
  - Growth at 7 – 40°C, optimum of 35-37°C
  - Poor survival at pH <4.5
- Generally regarded as fragile organisms
  - Cannot survive outside the intestinal environment
  - Wrong. They can survive and grow in different foods

Growth of Shigella on cubed papaya
From Escartin et al., J. Food Prot. 52:471, 1989

Shigellosis

- *S. dysenteriae* produces a neurotoxin called shiga toxin, which produces a severe syndrome
- Shigellosis consists of a dysenteric syndrome
  - Bacillary dysentery
  - Invasive virulence mechanism
- Incubation period of 7 h to 7 days
  - ~36 h in foodborne shigellosis
- Low infective dose
  - 10-100 cells
Foods associated with shigellosis in the U.S.

- Salads: 29%
- Seafood: 9%
- Mexican food: 7%
- Others: 18%
- Unknown: 37%

Methods of detection

- Enrichment in GN broth
- Plating in selective and differential media
  - XLD, McConkey, SS
- Biochemical and serological ID

*Yersinia enterocolitica*

- Genus *Yersinia*
  - *Y. pestis*
    - Bubonic plague
  - *Y. pseudotuberculosis*
    - Mesenteric adenitis
  - *Y. enterocolitica*
    - Gastroenteritis
**Y. enterocolitica**

- Characteristics of the organism
  - *Enterobacteriaceae* family
  - Psychrotroph
    - Grows From -1 to 40°C
    - Optimum 29°C
  - Motility depends on temperature
    - Motile at 25°C
    - Non-motile at 35°C
  - pH
    - Minimum 4.1 – 5.1 depending on acidulant
    - Optimum 7 - 8

**Y. enterocolitica**

- Isolated from different environments
  - Soil
  - Fresh water
  - Intestinal tract of many animals
- Has been found in many foods
  - Milk and milk products
  - Meats
  - Pork in particular
  - Poultry
  - Fish and shellfish
  - Fruits and vegetables

**Y. enterocolitica**

- Not a common disease in the United States
- Incubation period of 1 – 11 days
  - Lasts between 5 and 14 days
- Common syndrome includes self-limiting gastroenteritis
  - Fever
  - Abdominal pain
  - Sometimes mistakenly diagnosed as appendicitis
- Other forms of yersiniosis
  - Septicemia
  - Arthritis
**Y. enterocolitica**

- Foods associated with yersiniosis
  - Pork
  - Raw milk
    - A major outbreak in the U.S. was linked to contamination occurring during contact of milk crates with the environment of a pig farm
- Serotypes most commonly associated with human disease
  - O3, O5, 27, O8, O9
    - Frequently isolated from tongue, tonsils, nose and gut of pigs

**Y. enterocolitica**

- Isolation methods
  - Enrichment
    - Cold enrichment in a non-selective broth incubated for long time
    - Enrichment at 28°C in a selective medium
      - Added with cefsulodin, irgasan and novobiocin
    - CIN
  - Plating in a selective and differential medium
  - Biochemical identification
  - Serological and DNA characterization

**Y. enterocolitica**

- FDA method
  - Enrichment at 10°C in peptone sorbitol bile broth for 10 days
  - Transfer 1 ml into a tube with 9 ml of 0.5% KOH in 0.5% saline and agitate for 5 - 10 s
  - Spread over selective and differential plates and incubate at 30°C for 24 h
    - McConkey agar
    - CIN agar
  - ID tests
Microorganisms Causing Foodborne Disease

Enterobacteriaceae Family
Genus Enterobacter
Spices E. sakazakii

Enterobacter sakazakii

- Family: Enterobacteriaceae
- Genus: Enterobacter
  - Gram-negative rod
  - Motile (peritrichous)
  - Fermentative metabolism
  - Oxidase negative

Differentiation between E. sakazakii and E. cloacae

<table>
<thead>
<tr>
<th>Test:</th>
<th>E. sakazakii</th>
<th>E. cloacae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dulcitol</td>
<td>-</td>
<td>(-)</td>
</tr>
<tr>
<td>Adonitol</td>
<td>-</td>
<td>(-)</td>
</tr>
<tr>
<td>D-sorbitol</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>α-methyl-D-glucoside</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>D-arabitol</td>
<td>-</td>
<td>(-)</td>
</tr>
<tr>
<td>Yellow pigment</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Syndromes

- Meningitis
- Cerebral abscess
- Bacteremia
- Necrotic enterocolitis
- Urinary infections

Cases of *E. sakazakii* disease in different countries

<table>
<thead>
<tr>
<th>Age</th>
<th>No. patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td></td>
</tr>
<tr>
<td>#7 days</td>
<td>16</td>
</tr>
<tr>
<td>8 days–1 month</td>
<td>7</td>
</tr>
<tr>
<td>&gt;1 month</td>
<td>5</td>
</tr>
<tr>
<td>&gt;1–4 years</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>39–49</td>
<td>1</td>
</tr>
<tr>
<td>50–59</td>
<td>1</td>
</tr>
<tr>
<td>60–69</td>
<td>1</td>
</tr>
<tr>
<td>$70</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
</tr>
</tbody>
</table>

Adapted from Lai. Medicine, 80:113. 2001
Syndromes caused by *E. sakazakii* in infants

- Meningoencephalitis: 3%
- Urinary infection: 3%
- Enteritis: 3%
- Meningitis: 26%
- Bacteremia: 26%
- Meningitis + bacteremia: 39%

Adapted from Lai. Medicine, 80:113. 2001

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*E. sakazakii* disease

- **Infants**
  - Mostly newborns
  - Some between 2 and 4 years of age
  - Fatality rate:
    - With meningitis = 45%
    - Without meningitis = 33%
- **Adults**
  - Older than 60
  - Fatality rate: ~50%

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Countries where outbreaks have been reported

- Belgium
- Canada
- United States
- Greece
- Hollanda
- England
- Iceland
Foods Involved

- Only one type of food product:
- Infant formula

Isolation of *E. sakazakii* from samples of baby formula

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>No. samples</th>
<th>% +</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>1997</td>
<td>120</td>
<td>6.7</td>
<td>Nazarwec-White &amp; Farber. J. Food Prot. 60:226</td>
</tr>
</tbody>
</table>

- Samples incoming from 35 countries
- *E. sakazakii* counts ranged from 0.4 to 66 MPN/100 g

Temperature limits for growth of *E. sakazakii* in BHI broth

<table>
<thead>
<tr>
<th>Origin</th>
<th>Isolate No.</th>
<th>Growth temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowest</td>
<td>Highest</td>
</tr>
<tr>
<td>Clinical isolates</td>
<td>1</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.5</td>
</tr>
<tr>
<td>Low range 5.5 – 8.0</td>
<td>6.0</td>
<td>43</td>
</tr>
<tr>
<td>Mean 6.4</td>
<td>5.5</td>
<td>42</td>
</tr>
<tr>
<td>Food isolates</td>
<td>1</td>
<td>7.5</td>
</tr>
<tr>
<td>Low range 5.5 – 7.5</td>
<td>5.5</td>
<td>43</td>
</tr>
<tr>
<td>Mean 6.3</td>
<td>6.0</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>42</td>
</tr>
</tbody>
</table>

D values of *E. sakazakii* in reconstituted infant formula stored at different temperatures

![Graph showing D values of *E. sakazakii*](image)


<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Log reduction (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>67.5</td>
<td>1</td>
</tr>
<tr>
<td>68</td>
<td>2</td>
</tr>
</tbody>
</table>

Simulated reductions of *E. sakazakii* and *L. monocytogenes* obtained by using @RISK™ modeling package.

Model simulated reduction after 16 s of contact

![Graph showing simulated reductions](image)

Adapted from Nazarowec-White et al. Food Res. Int. 32:375. 1999

Incidence of *E. sakazakii* in food samples collected from university cafeterias. Valencia, Spain

Soriano et al. Food Microbiol. 18:159. 2001

<table>
<thead>
<tr>
<th>Food</th>
<th>Preparation</th>
<th>No. samples</th>
<th>No. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce</td>
<td>Fresh</td>
<td>40</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td></td>
<td>RTE</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Pork</td>
<td>Raw</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>RTE</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Beef</td>
<td>Raw</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>RTE</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Poultry</td>
<td>Raw</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>RTE</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Spanish tortilla</td>
<td>RTE</td>
<td>40</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

(egg omelet)
Insects as potential reservoirs

<table>
<thead>
<tr>
<th>Insect</th>
<th>Species</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>House fly</td>
<td>Musca domestica</td>
<td>Citado por Hamilton et al., 2003</td>
</tr>
</tbody>
</table>

Virulence studies

Enterotoxin production in *E. sakazakii* isolates determined by the suckling mouse test

<table>
<thead>
<tr>
<th>Origin of isolates</th>
<th>No. isolates tested</th>
<th>No. isolates with positive reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Foods</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

Heated and unheated extracts in 1 of 3 *E. sakazakii* isolates showed reaction with CHO, Vero and Y-1 cells

- Dose to 100% lethality in mice: 10\(^8\) célis
- Dose to 100% survival: 10\(^4\) células

Control

Appropriate heat treatment
- Dairy industry
  - Avoid spray drying of milk
- Infant feeding
  - Retort bottles with formula before feeding newborns
  - Apply good manufacturing practices to prevent recontamination
  - Refrigerate unused bottles
Food testing for *E. sakazakii*

**FDA. Isolation and enumeration of *E. sakazakii* in dry baby formulae**

- **Presumptive test**
  - Weigh 3 x 100, 10 and 1 g of product in 2 L, 250 and 125 ml Erlenmeyer flasks
  - Add 9 parts (1:10 dil.) sterile distilled water at 45°C and stir to mix then incubate overnight at 36°C with cap loosened
  - Transfer 10 ml of this suspension into 90 ml EE broth in sterile 150 ml bottles and incubate overnight at 36°C
  - Prepare serial dilutions and surface spread over violet red bile glucose (VRBG) agar plates. Incubate
  - Plate by streaking on VRBG. Incubate

**Confirmation test for *E. sakazakii***

- **5 suspicious colonies**
- **Streak isolate on TSA**
- **If API tests are positive**
  - **Yellow colonies**
  - **Confirm by oxidase test**
- **Confirm using API 20E**

**Real time PCR methods**

- **FDA**
  - Primers and detectors
    - Partial macromolecular synthesis operon
    - Gen rpsU, extreme 3' and from primase (dnaG), extreme 5'
    - High specificity against other *Enterobacter* spp.
    - Detects 10^2 UFC/ml
    - In pure cultures and in dairy formula
    - 50 PCR cycles
    - No enrichment
- **Bax® (DuPont) + Nestlé**
  - Designed for samples of dry infant formula, dairy ingredients, soybean ingredients and environmental samples
  - Results in 24 h
  - 99% specificity
  - High sensitivity