Enterobacter sakazakii in Powdered Infant Formula

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Outline

- The organism/disease: *E. sakazakii*/meningitis, septicemia
- The product: powdered-infant formula (PIF)
- Risk assessment:

  ALOP? → FSO? → PO? → MC
Enterobacter sakazakii

- Gram-negative rod; Enterobacteriaceae family
- Non-spore forming, motile
- 1980, designated as a unique species based on differences from *E. cloacae* in DNA relatedness, pigment production and biochemical reactions (sorbitol, α-glucosidase)
Enterobacter sakazakii

- 14 or 15 Biogroups
- Biochemical reactions; motility
- Produces a capsule
- Genetically diverse (4 clusters; 2 lineages described to-date)
E. sakazakii infections

- About 90 cases worldwide to-date, 80% of them in infants < 1 y old
- Among infants, 66% of cases are 0-1 month of age and 14% > 1 month – 1 year
- **Conclusion:** Group at particular risk is infants (<1 year; 4-6 months)
- **Greatest risk:** neonates (<28d) and immunocompromised, especially those of LBW (<2500 g)
Hazard Characterization

• **Symptoms**
  – Meningitis, septicemia, NEC, death possible, chronic sequelae

• **Dose response**
  – Unknown
  – Likely depends on age/weight of patient/health status
Environmental sources of *E. sakazakii*

- Dust
- Fruit flies, house/stable flies
- Rats
- Soil, rhizosphere
- Sediment, wetlands
Foods from which the organism has been isolated

- Cured meat, minced beef, sausages
- Lettuce, vegetables, alfalfa sprouts
- Tofu; bread, cheese; rice seed
- Herbs & spices
- Sous (licorice drink)
- Dried products (infant cereal, veggies, spices, whey, egg yolk/eggnog, flour/meal)
- Mother’s milk
### Occurrence in food production environments and households

<table>
<thead>
<tr>
<th>Site</th>
<th>Samples positive for <em>E. sakazakii</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk powder factory</td>
<td>14/68 (21)</td>
</tr>
<tr>
<td>Chocolate factory</td>
<td>2/8 (25)</td>
</tr>
<tr>
<td>Cereal factory</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>Potato flour factory</td>
<td>4/15 (27)</td>
</tr>
<tr>
<td>Pasta factory</td>
<td>6/25 (23)</td>
</tr>
<tr>
<td>Households</td>
<td>5/16 (31)</td>
</tr>
</tbody>
</table>

Kandhai et al., 2004; Lancet
Powdered infant formula (PIF)

Formulations can contain:
- Milk ingredients and/or corn syrup solids
- Fats, sugars, starch
- Vitamins/minerals

PIF is given to:
- In general, infants under 6 months
- Low and very-low-birthweight newborns
- Newborns/infants with special nutritional needs
- Infants of mothers with nutritional or health problems (e.g., HIV positive)
### Outbreaks linked to PIF

<table>
<thead>
<tr>
<th>Location</th>
<th>Cases</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceland</td>
<td>3 (1 death)</td>
<td>2 normal term infants; 1 Down's</td>
<td>Biering et al., 1989</td>
</tr>
<tr>
<td>Tennessee</td>
<td>4; 3 sepsis, 1 bloody diarrhea</td>
<td>Es; 8 cfu/100g</td>
<td>Simmons et al., 1989</td>
</tr>
<tr>
<td>Belgium</td>
<td>12</td>
<td>6/12 with NEC positive for Es</td>
<td>Van Acker et al., 2001</td>
</tr>
<tr>
<td>Tennessee</td>
<td>9</td>
<td>1 confirmed, 2 suspect, 6 colonized</td>
<td>Himelright et al., 2001</td>
</tr>
<tr>
<td>Israel</td>
<td>5</td>
<td>3 colonized only</td>
<td>Bar-Oz et al., 2001</td>
</tr>
<tr>
<td>New Zealand</td>
<td>5 (1 death)</td>
<td>4 colonized</td>
<td>2004</td>
</tr>
<tr>
<td>France</td>
<td>9 (2 deaths)</td>
<td>5 colonized</td>
<td>AFSSA, 2005</td>
</tr>
</tbody>
</table>
### Incidence of *E. sakazakii* in PIF

<table>
<thead>
<tr>
<th>Samples positive (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/141 (14)</td>
<td>Muytjens (1988)</td>
</tr>
<tr>
<td>8/120 (6.7)</td>
<td>N-White &amp; Farber (1997)</td>
</tr>
<tr>
<td>8/210 (3.8)</td>
<td>Heuvelink et al. (2001)</td>
</tr>
<tr>
<td>3/141 (2.1)</td>
<td>Heuvelink et al. (2003)</td>
</tr>
<tr>
<td>8/58 (13.8)</td>
<td>Leuschner et al. (2004)</td>
</tr>
<tr>
<td>35/3,467 (1.0)</td>
<td>IFC (2004)</td>
</tr>
<tr>
<td>1/835 (0.12)</td>
<td>WHO (2004)</td>
</tr>
</tbody>
</table>
Production methods for PIF

Raw Ingredients
- Wet blend
- Dry blend
- Heat
- Dry

PIF
- Home Preparation
- Hospital Preparation

Consumption

WHO, 2004
Sites of potential contamination

- Raw milk
- Transport
- Storage
- Pasteurization
- Drying/Blending
- Filling Product
- Storage
- Transport
- Reconstitution
- Consumption
- E sak

Potential sites for Environmental contamination
Reduction

Farm

- Raw milk
- Transport
  - Storage
- Pasteurization
- Drying/
  - Blending

Factory

- Filling
  - Product
- Storage
  - Transport
- Reconstitution
- Consumption

Hospital/
  - Home

E sak

< 1 cfu /10^8g  Σ R = -8
Increase through recontamination and growth

Farm

Raw milk
Transport Storage
Pasteurization
Drying/Blending
Filled Product
Storage Transport
Reconstitution Consumption

Factory

Hospital/Home

E sak
Unknown; $\Sigma I = ?$

Increase through recontamination and growth
### E. sakazakii – Growth in PIF

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Lag time</th>
<th>Gen. time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6°C</td>
<td>ND</td>
<td>13.7 h</td>
<td>Iversen et al. (2004)</td>
</tr>
<tr>
<td>10°C</td>
<td>19-47 h</td>
<td>4.2-5.5 h</td>
<td>N-White &amp; Farber (1997)</td>
</tr>
<tr>
<td>21°C</td>
<td>ND</td>
<td>1.7 h</td>
<td>Iversen et al. (2004)</td>
</tr>
<tr>
<td>23°C</td>
<td>1.8-3.4 h</td>
<td>37-44 min</td>
<td>N-White &amp; Farber (1997)</td>
</tr>
<tr>
<td></td>
<td>3.9-4.7 h</td>
<td>43 min</td>
<td>Lenati (2005)</td>
</tr>
<tr>
<td>37°C</td>
<td>ND</td>
<td>19-21 min</td>
<td>Iversen et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>2.2-3.0 h</td>
<td>17.4 min</td>
<td>Lenati (2005)</td>
</tr>
</tbody>
</table>

ND=Not Determined
Key Factors Affecting the Risks Associated with PIF

- Initial level of contamination
- Level of hygiene in the preparation & delivery of the rehydrated formula
- Bactericidal treatment at time of preparation
- Duration of time to consumption and holding/storage temperature

FAO/WHO, 2004
Relative risk of *E. sakazakii* infection for specified holding time and temps*  

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Room Temperature ( °C ) - liquid mixing temp 40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15°C</td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>3</td>
<td>4.8</td>
</tr>
<tr>
<td>4</td>
<td>8.2</td>
</tr>
<tr>
<td>8</td>
<td>47.3</td>
</tr>
</tbody>
</table>

*Risk estimates relative to est. risk at 1 h of holding at each temp*  

Source: Decisionalysis Risk Consultants, 2004
Control measures for *E. sakazakii* in PIF

Reducing the concentration/prevalence of intrinsic contamination

\[ H_0 - \Sigma R + \Sigma I \leq FSO \]

Reducing the level of contamination of the reconstituted PIF (e.g., heat) prior to use

Minimise the chance of contamination of reconstituted formula during preparation

Minimize the growth of *E. sakazakii* following reconstitution prior to consumption
Food Safety Objective (FSO) and Performance Objective (PO)

PO – at packaging

FSO

subtract growth

Reconstitution, Storage and/or Holding Times
Hypothetical example in establishing Performance Objective

• FSO of < 1 CFU/day
• LBW infants receive 150 mL liquid formula per kg bodyweight/day
• 0.216 g PIF / mL
• Example: 2.5 kg infant receives 81 g PIF / day and so FSO would be an absence in 81 g (−1.9 log CFU/g)
### Scenario 1: 12 h at 10°C; 3.3 h at RT

<table>
<thead>
<tr>
<th>Birthweight (kg)</th>
<th>FSO (log cfu/g)</th>
<th>Predicted growth (log cfu/g)</th>
<th>PO (log cfu/g)</th>
<th>Absence in (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>-1.51 (absence in 33g)</td>
<td></td>
<td>-2.11</td>
<td>129</td>
</tr>
<tr>
<td>1.50</td>
<td>-1.69</td>
<td></td>
<td>-2.29</td>
<td>193</td>
</tr>
<tr>
<td>2.00</td>
<td>-1.81</td>
<td>0.60</td>
<td>-2.41</td>
<td>258</td>
</tr>
<tr>
<td>2.50</td>
<td>-1.91 (absence in 81g)</td>
<td></td>
<td>-2.51</td>
<td>322</td>
</tr>
</tbody>
</table>

Calculation e.g., PO = -2.11 log cfu/g = absence in $1/(10^{-2.11})$ g = 129 g
Product / Pathogen / Pathway of *E. sakazakii* in PIF

Log CFU/ g

Absence in 322g

Absence in 81 g (for 2.5 kg bw)

PO

FSO

Reception

Heating

Packaging/ Reconstitution

Consumption

$H_0$

$IRC$

$IRC+G$

8 D
Selection and calculation of POs and other limits

Scenario 2: 12 h at 10°C; 1 h at 37°C; 3.3 h at RT

<table>
<thead>
<tr>
<th>Birthweight (kg)</th>
<th>FSO (log cfu/g)</th>
<th>Predicted Growth (log cfu/g)</th>
<th>PO (log cfu/g)</th>
<th>Absence in Absence in (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>-1.51</td>
<td></td>
<td>-3.02</td>
<td>1048.4</td>
</tr>
<tr>
<td>1.50</td>
<td>-1.69</td>
<td></td>
<td>-3.20</td>
<td>1572.7</td>
</tr>
<tr>
<td>2.00</td>
<td>-1.81</td>
<td>1.51</td>
<td>-3.32</td>
<td>2096.9</td>
</tr>
<tr>
<td>2.50</td>
<td>-1.91</td>
<td></td>
<td>-3.42</td>
<td>2621.1</td>
</tr>
</tbody>
</table>

Hypothetical example
Selection and calculation of POs and other limits

**Scenario 5: 8 h at 30°C (e.g., developing country)**

<table>
<thead>
<tr>
<th>Birthweight (kg)</th>
<th>FSO (log cfu/g)</th>
<th>Predicted growth (log cfu/g)</th>
<th>PO (log cfu/g)</th>
<th>Absence in (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>-1.51</td>
<td></td>
<td>-5.12</td>
<td>132</td>
</tr>
<tr>
<td>1.50</td>
<td>-1.69</td>
<td></td>
<td>-5.30</td>
<td>198</td>
</tr>
<tr>
<td>2.00</td>
<td>-1.81</td>
<td>3.61</td>
<td>-5.42</td>
<td>264</td>
</tr>
<tr>
<td>2.50</td>
<td>-1.91</td>
<td></td>
<td>-5.52</td>
<td>330</td>
</tr>
</tbody>
</table>

Hypothetical example
Microbiological Criteria - *E. sakazakii*

- **Farm**
  - Raw milk
  - Transport
  - Storage
- **Factory**
  - Pasteurization
  - Drying/Blending
  - Filled Product
  - Storage
  - Transport
  - Reconstitution
  - Consumption
- **Hospital/Home**
  - Filled Product

**Authorities**
- Codex (draft) < 0.33 CFU/100 g
- Thailand < 0.06 CFU/100 g
- U.S. < 0.075 – 0.3 CFU/100g
- EU < 0.33 CFU/100 g
ICMSF/Codex Criteria for E. sakazakii

- 2-class sampling plan
- N=30; c=0; m=0/10g
- Assuming
  - Log normal distribution
  - SD 0.8
- Plan would have a 95% probability of rejecting a lot with a mean level of 1 cfu/112g
Microbiological Criteria

Farm
- Raw milk
- Transport
- Storage
- Pasteurization
- Drying/Blending

Factory
- Filled Product
- Storage
- Transport
- Reconstitution
- Consumption

Hospital/Home

Manufacturer

E. sakazakii:
- Environment
- In-line
- Finished product
Many individuals unaware that PIF is not a sterile product
Lack information on how handling, storage and preparation practices can influence the risk
Effective risk communication practices needed for the public and health professionals
Some publications are available
Summary

• *E. sakazakii* infection is a rare, but very serious disease
• More data needed on true “susceptible” populations
• More growth data needed
• Dose-response models needed to develop FSOs
• More global data needed on PIF handling, preparation and storing practices
Future Work

• ICMSF – criteria/specifications
• ILSI grant to look at animal models
• Risk assessment (FAO/WHO)
Thank you very much!