Cronobacter sakazakii

NM

1/04/2021

Content

- Characteristics
- Diseases
- Virulence factors
- Source of contaminat
- Prevention & control
- Outbreaks

Cronobacter sakazakii

- Gram-negative, peritrichously flagellated, motile, rod-shaped, non-sporulating pathogens, facultative anaerobic
- Opportunistic pathogens – able to become pathogenic when the host's immune system is impaired / weak
- Previously known as "yellow pigmented Enterobacter cloacae"
- Re-classified in 1980s as *Enterobacter sakazakii*
- Changed to *Cronobacter sakazakii* in 2008 – advancement in identification method such as 16s ribosomal DNA, hsp60 sequencing and polyphasic analysis
- Sakazakii – Japanese microbiologist, Riichi Sakazaki (1920-2002), was a bacterial taxonomist involve in the nomenclature.

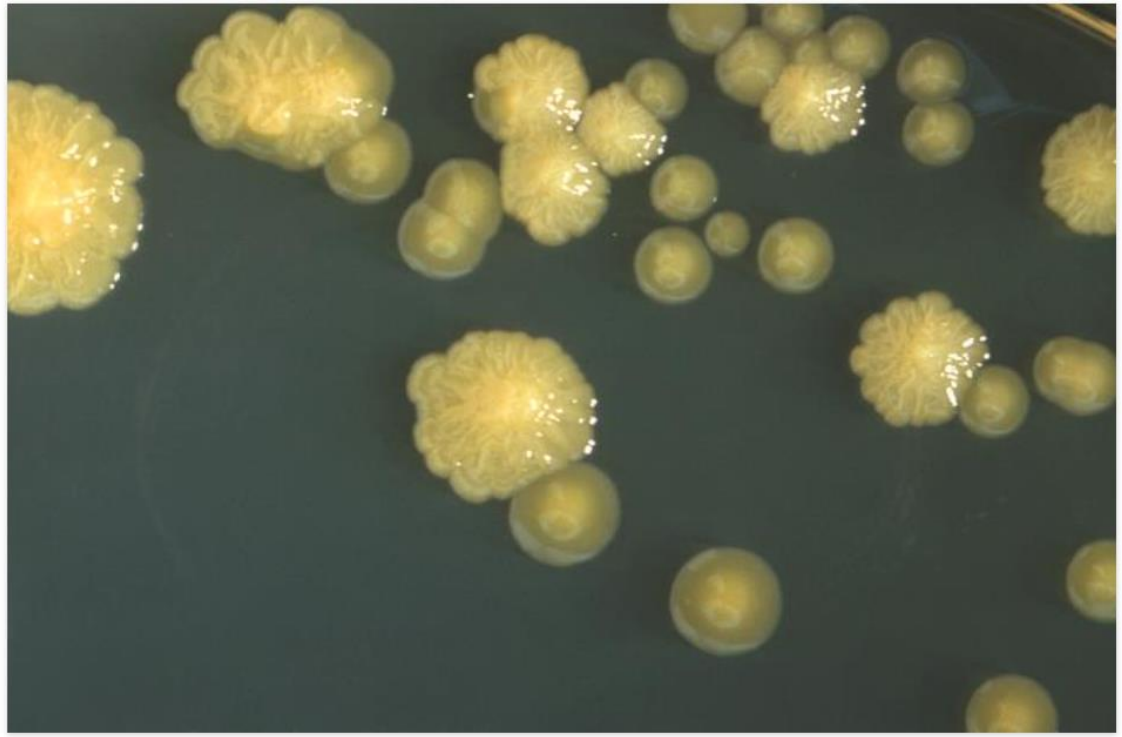


Figure 1. Bacterial colonies of C. sakazakii on a Petri dish after a three day incubation at 25 °C on trypticase soy agar. Source: Public Health Image Library, Center for Disease Control, Dr. J.J. Farmer (1978).

Cronobacter sakazakii

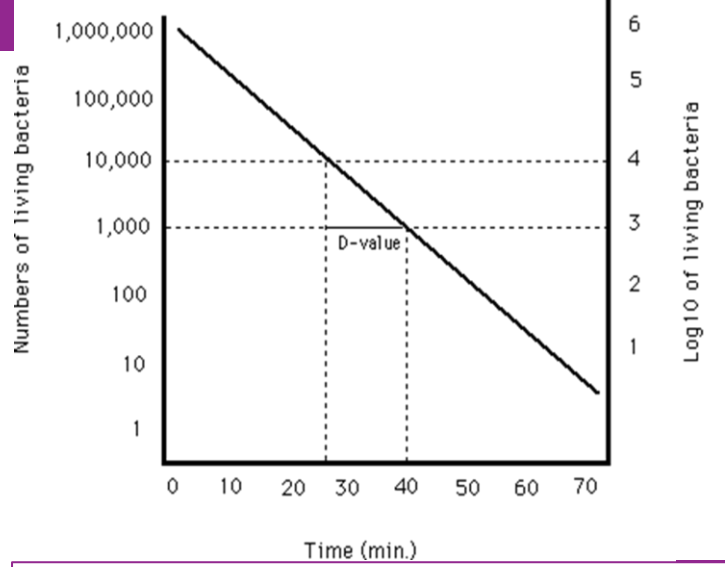
- Capable of surviving in dry environment. E.g. powdered infant formula, cereals, dried herbs, pasta.
- Powdered infant formula is the most common. Traced in:
  - Freshly prepared or refrigerated powdered milk
  - Utensils & equipment used in formula preparation
  - Reconstitute product
- Cross contamination – manufacturing facilities, healthcare settings, domestic environments

Cronobacter sakazakii

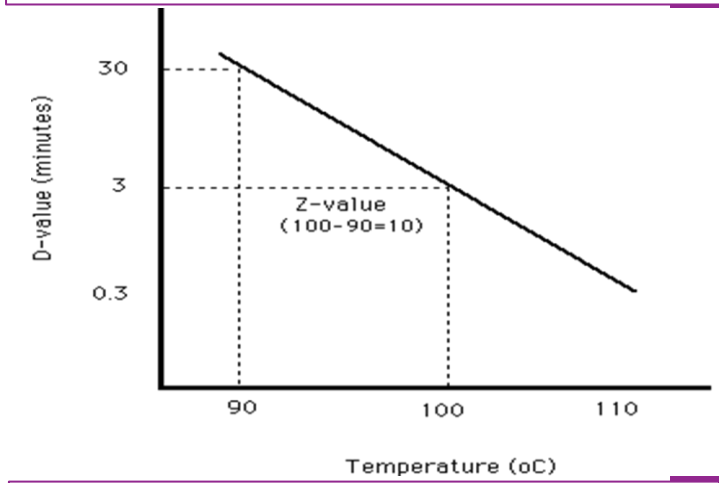
- Infant are more susceptible due to their weak immune system.
- *Cronobacter* infections are **rare** (4-6 infections/year), but they can be **deadly in newborns** (infants < 2 months / premature babies are at the highest risk)
- Infection in infants usually occur in the first days or weeks of life.
- Infective dose: ~ 10 -100 organisms.
- Onset: Symptoms occur in infants within few days.
- Duration of symptoms: Among survivor (2 -8 weeks), Among fatalities (death may occur within few hours to several days after 1st sign of sepsis)
- Mortality/Death rate: **40 – 80%**

GROWTH

- Wide growthT:as low as 5.5 – 45 °C
- OptimalT:34 – 37°C
- Warm water (52 -58 °C) can reduce the number of pathogen in reconstituted powdered infant milk but *C. sakazakii* will be fully inactivated at temp 70°C
- One of thermotolerant bacteria, higher than most Gram negative bacteria.
  - $D_{60°C}$ = 2.5 – 3.06 min
  - $D_{58°C}$ up to 9.9 min
  - (z = 5.82 °C)
- Criteria unique to *Cronobacter sakazakii* is the ability to utilize **sialic acid** (additive in infant formula) for growth.

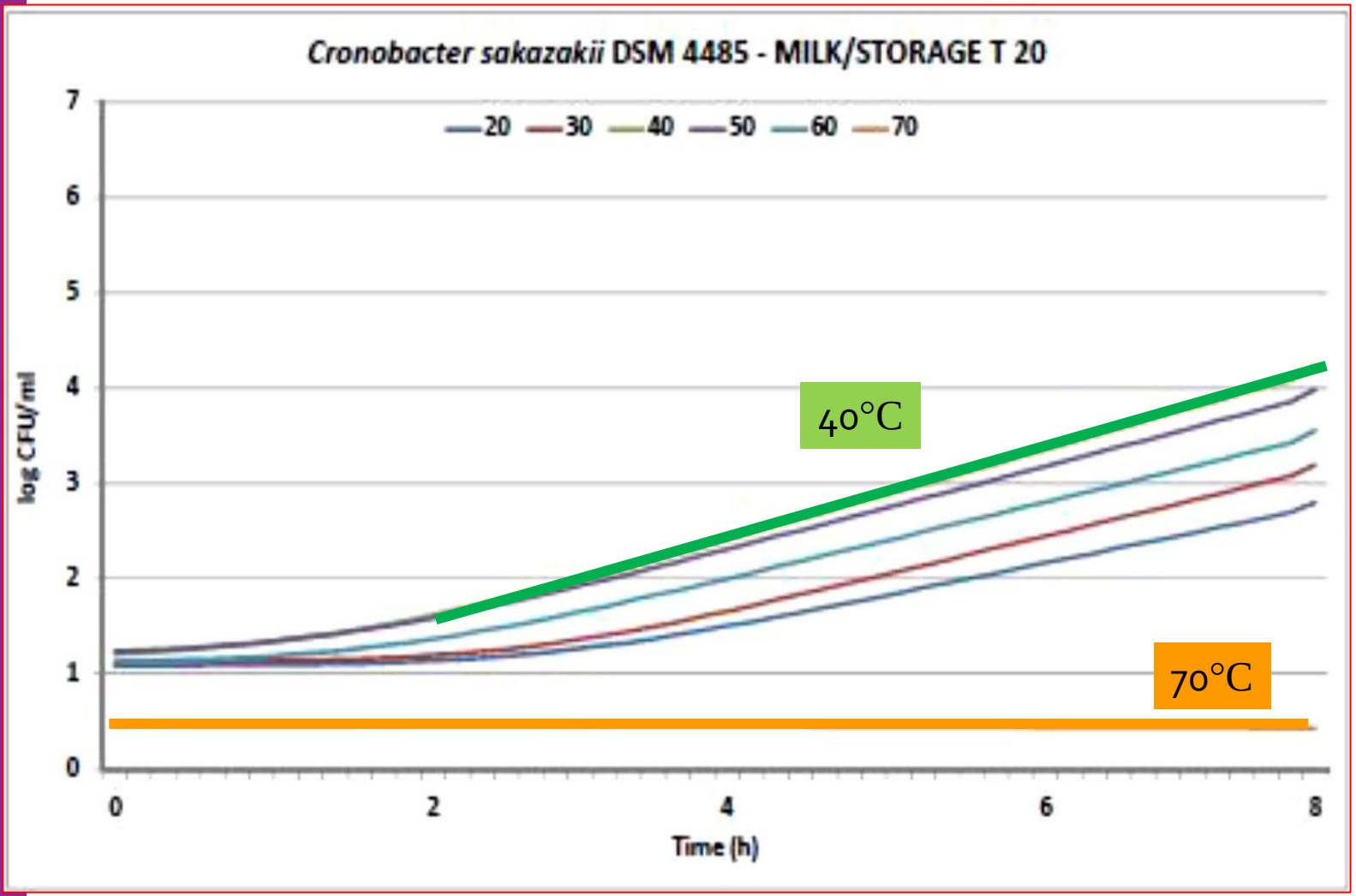


D-value is defined as the time required at temperature (T) to reduce a specific microbial population by 90% (1 log reduction).



Z-value is defined as the temperature change required to change the D value by a factor of 10

Effect Of **Reconstitution** & **Storage Temperatures** On Growth Of ***C. Sakazakii*** In **Powdered Infant Formula**



Storage of **rehydrated formulas** at room temperatures promote growth, especially if the **reconstitution Temp.** is close to the optimal growth range (40 °C).

70 °C = inactivation

EVA MARÍA HERNÁNDEZ QUESADA (2012) UNIVERSITY AUTONOMOUS OF BARCELONA

How does powdered infant formula become contaminated with *Cronobacter*?

- Source of contamination:
  - Contaminated raw materials used to make the formula
  - Contaminated surface/ equipment's in the factory
  - Contaminated at home after the container is opened

- Powdered infant formula is not sterile.
  - Nutritional value must be in compliance with the regulatory standard
  - Nutrients are heat-labile (vitamins, minerals, amino acids, fatty acids) and must be added after pasteurization to avoid denaturation.
  - *C. sakazakii* can be introduced into the infant formula

Manufacturing process of powdered infant formula

❑Dry-blending

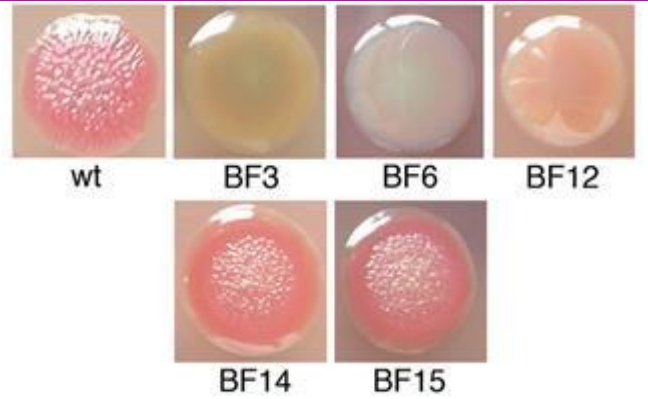
- Dehydrated powdered ingredients are blended in large batches until nutrient/ingredients are distributed uniformly.
- Pass through sifters to removed oversized particles and foreign materials.
- Flushed with inert gas, sealed, labelled, coded, and packaged in carton. Final microbiological check for finished product
- Disadvantage: **No thermal processing**

❑Wet-blend spray drying

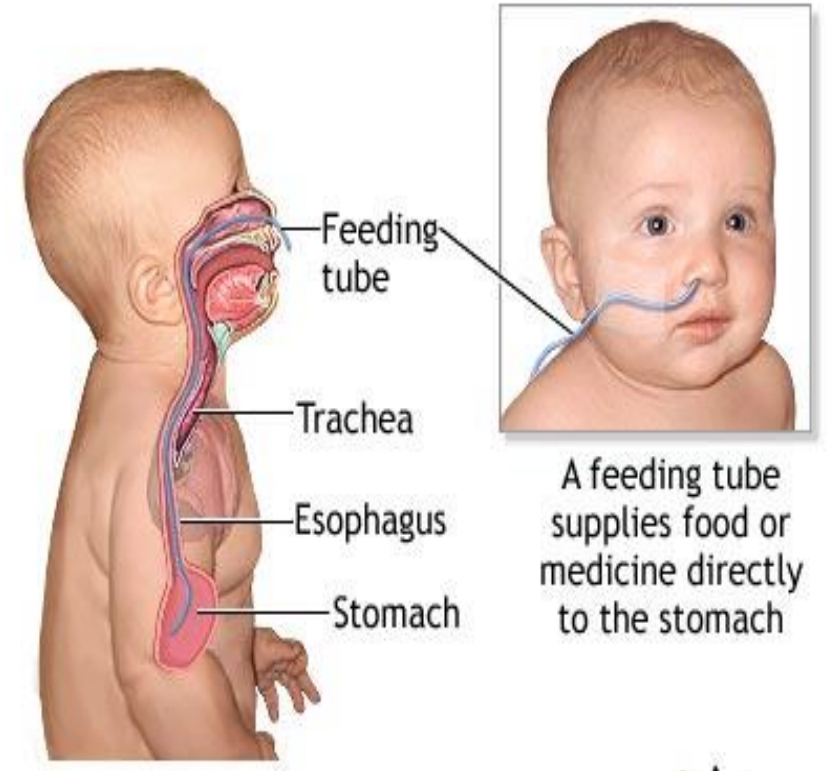
- Pasteurization of milk
- Homogenisation
- Addition of heat-sensitive micronutrients
- Pass through evaporator that is heated up to 62-77°C & transferred through a high-pressure pump to spray dryer nozzles or cooled for storage, then, reheated and pump directly to the spray dryer
- Water is evaporated in the spray dryer, dry powder is created at the bottom of the dryer (73-79°C)
- Cooled by a stream of chilled filtered air and passed through a sifter for packaging.
- Final microbiological check
- Disadvantage: It contains **water in its processing** and has a higher chance of the proliferation of pathogenic bacteria

SURVIVAL CHARACTERISTICS

- Has the ability to form **biofilms** on **biotic and abiotic surfaces** that is resistant to drying and osmotic stress
  - Biofilm – a thick layer of bacterial cells that have aggregated to form a colony, adhered to a surface and coated with polysaccharide layer (slime layer)
  - The slime layers aid in protecting the bacteria, promote growth and survival
  - Biotic surface – infant formula, human intestines
  - Abiotic surface – stainless steel utensils, polyester plastic
- *C. sakazakii* can form biofilm on the feeding tube of neonates
- **Survival in dry environments** & **low-moisture food** (aw 0.30 – 0.83) forextended periods of time (>**2 years** in dry food products)
- **Acid tolerant**: resistant to low pH environment & human stomach.
- **Thermotolerance; antibiotic resistance – amphicillin, cefotaxime,cephalotin, ceftriaxone**



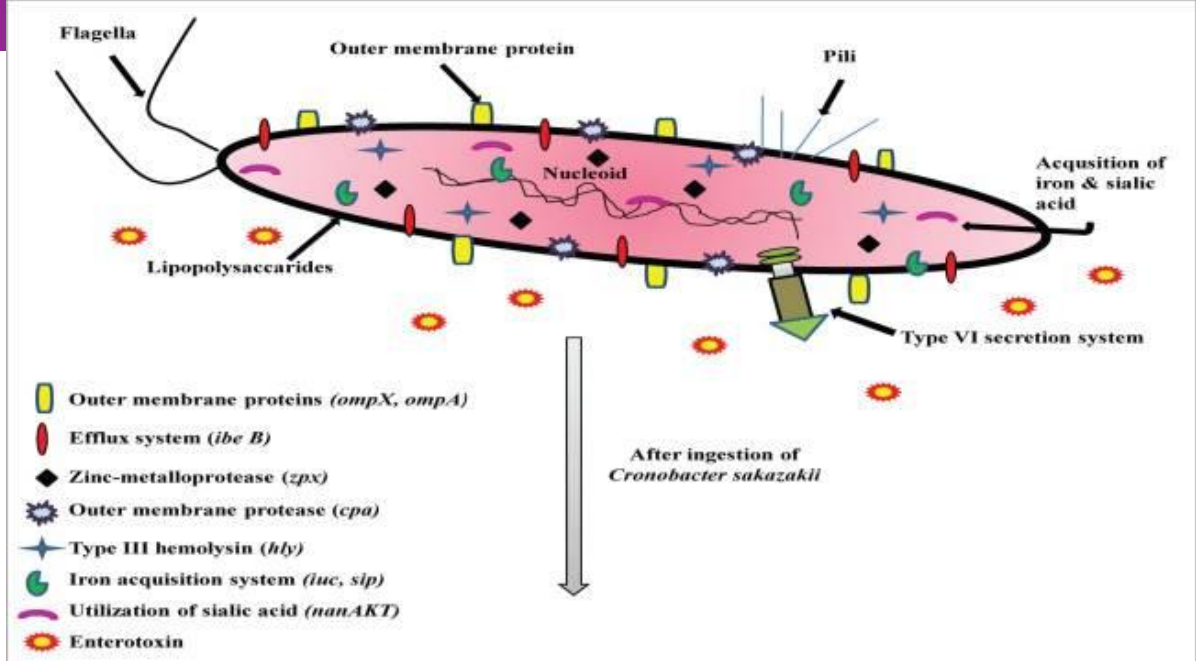
Biofilm formation of different strains.

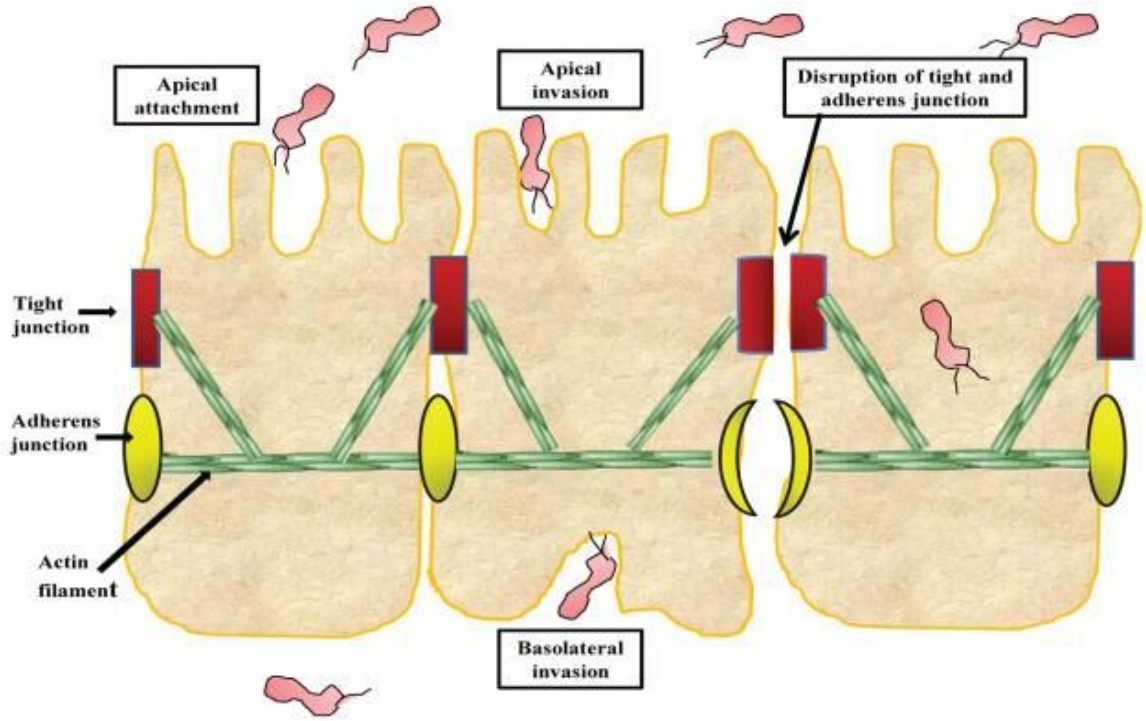


http://aem.asm.org/content/76/7/2251/F2.expansion.html

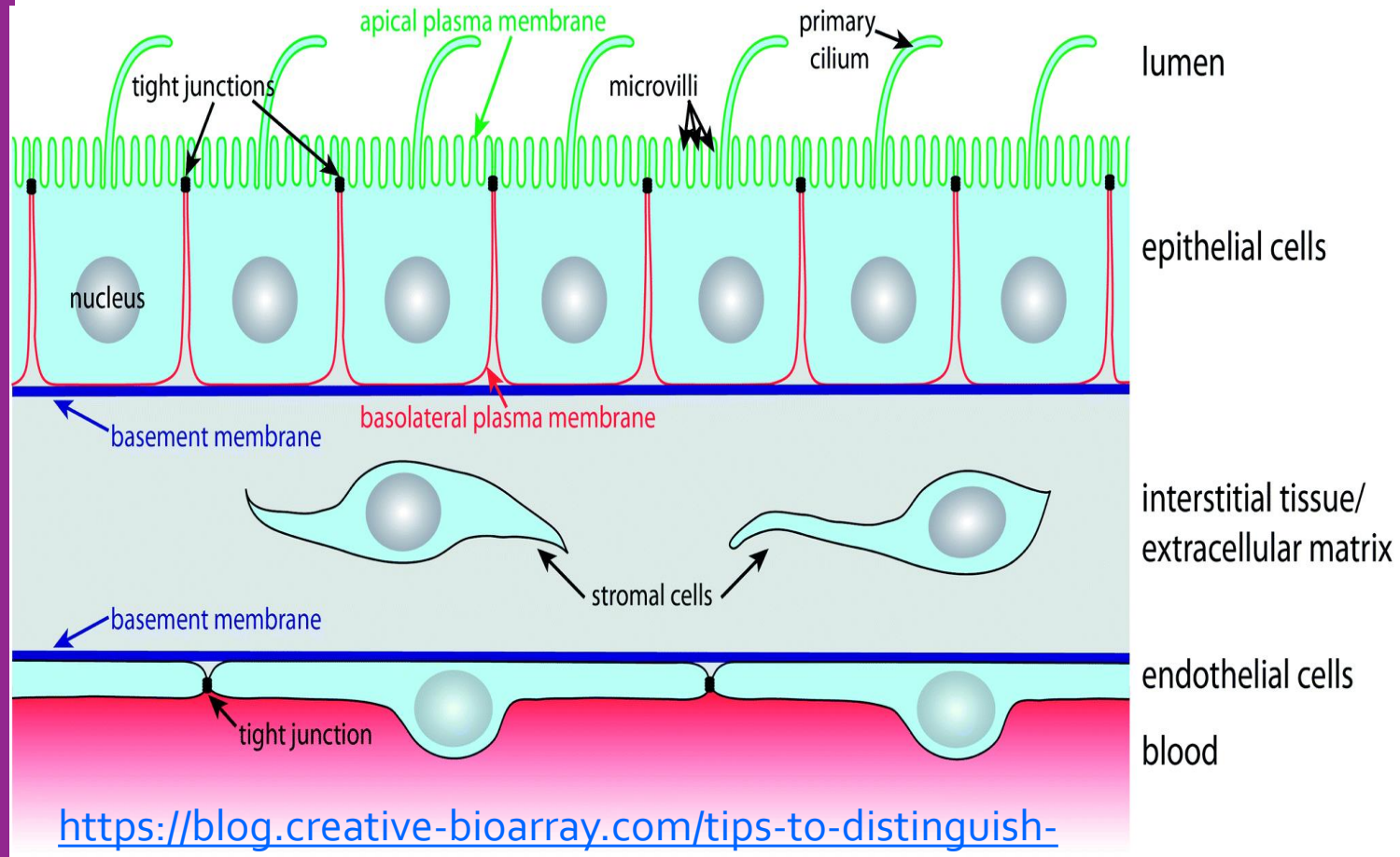
Virulence factors

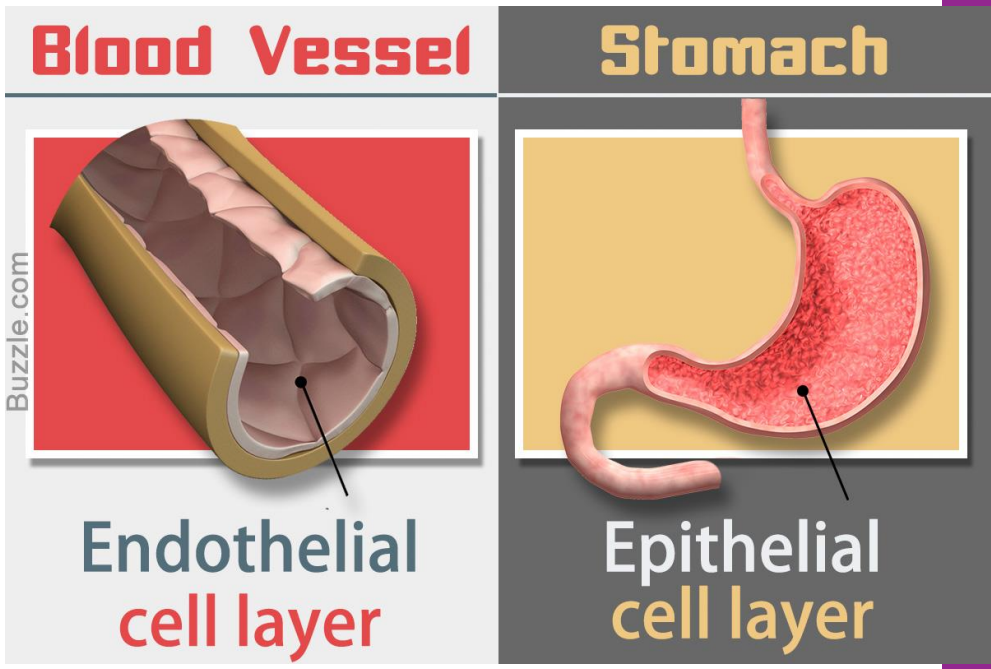
- **Sialic acid utilization** – Sialic acid is found in huma milk and infant formula. *Cronobacter sakazakii* is the only species that possess the *nanAKT* gene cluster encoding for sialic acid utilization.
- **Enterotoxins** - Some strain produce heat-stable enterotoxin that able to survive pasteurization
- **Efflux system** – *ibeB* gene in *C. sakazakii* is responsible in the invasion of brain microvascular endothelial cells. Also remove antibiotic from cytoplasm
- **Lipopolysaccharide** – disrupt the epithelial tight junction & facilitates the translocation of the bacteria across the intestinal wall
- **Outer membrane protein (OMPs)** – *ompA* invades various epithelial and endothelial cells; breach the blood-brain barrier and invade central nervous system. *ompX* invade both the apical and the basolateral side of host cell and translocate into the deeper organs





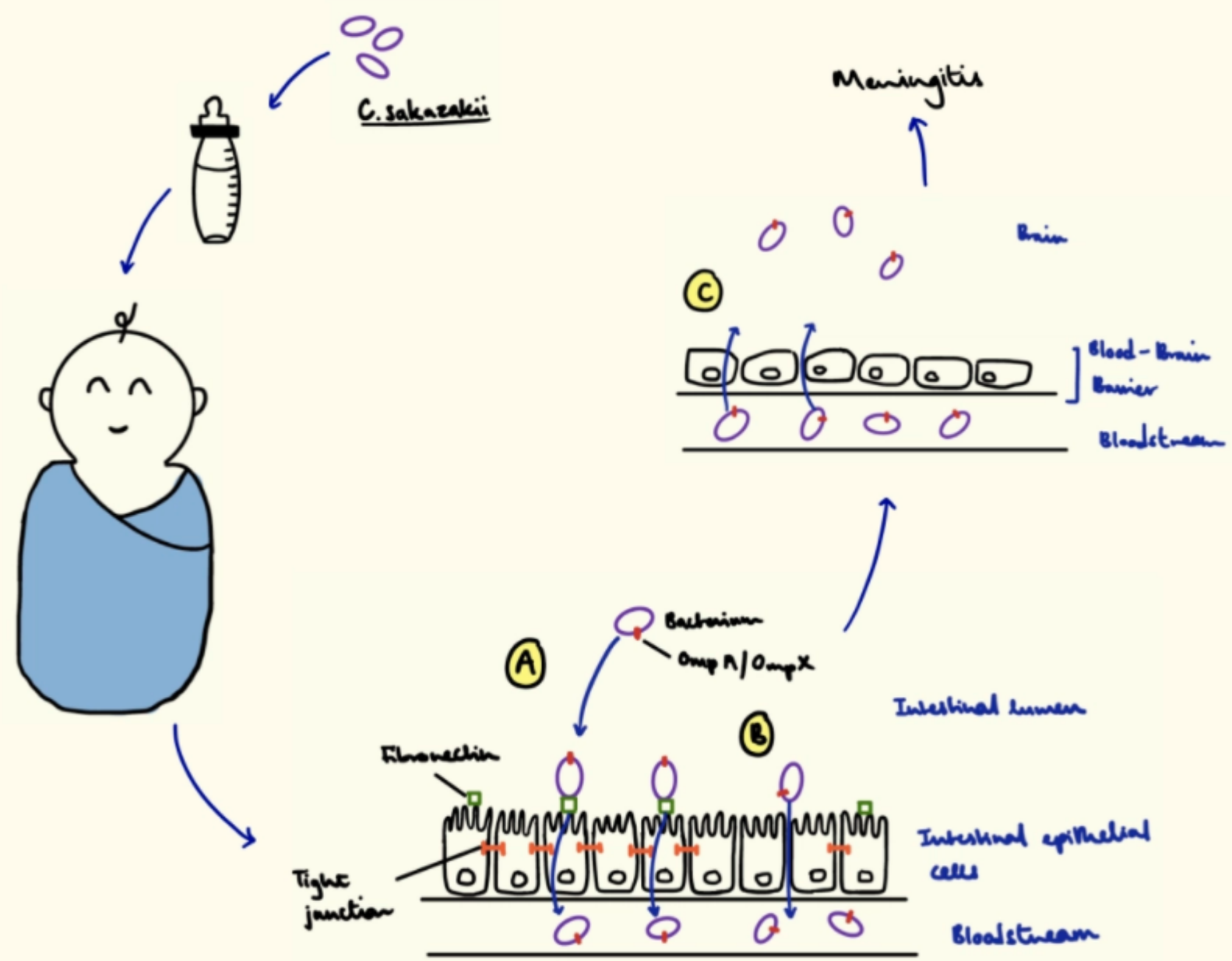
Epithelial and endothelial cells





https://blog.creative-bioarray.com/tips-to-distinguish-epithelial-cells-and-endothelial-cells/

PATHOGENESIS



A: Bacterium adheres to the fibronectin on the intestinal epithelial cells.
- OmpA & OmpX invade the cells
-get into the bloodstream

B. It can also bypass the underdeveloped tight junction (between cells) – bloodstream

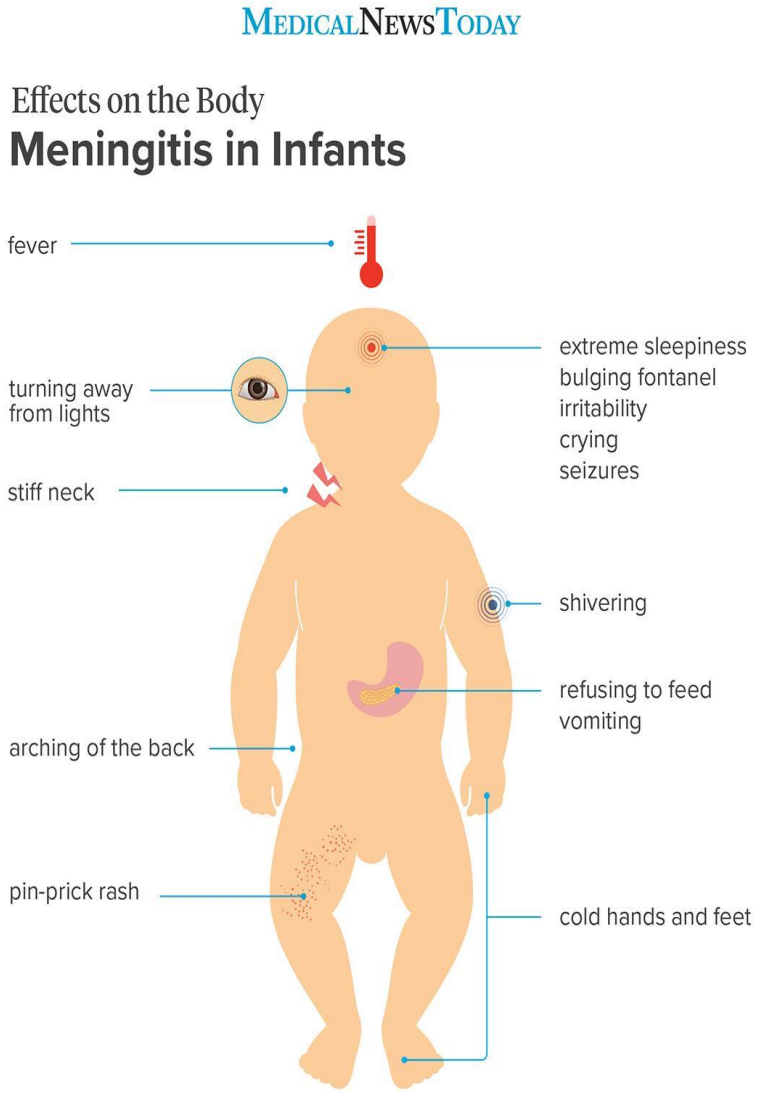
C: Travel to the blood-brain barrier & infecting the endothelial cells (barrier) --> cause meningitis (inflammation of the membrane)

https://mechpath.com/2019/12/03/cronobacter-sakazakii/

Diseases caused by *Cronobacter* infections

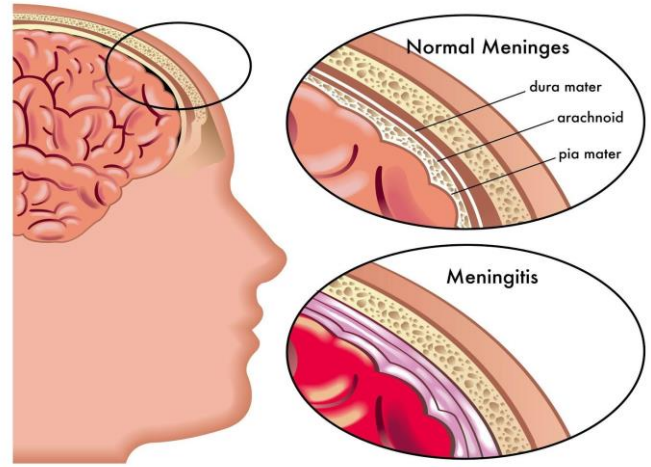
- Babies (less than 2 months)

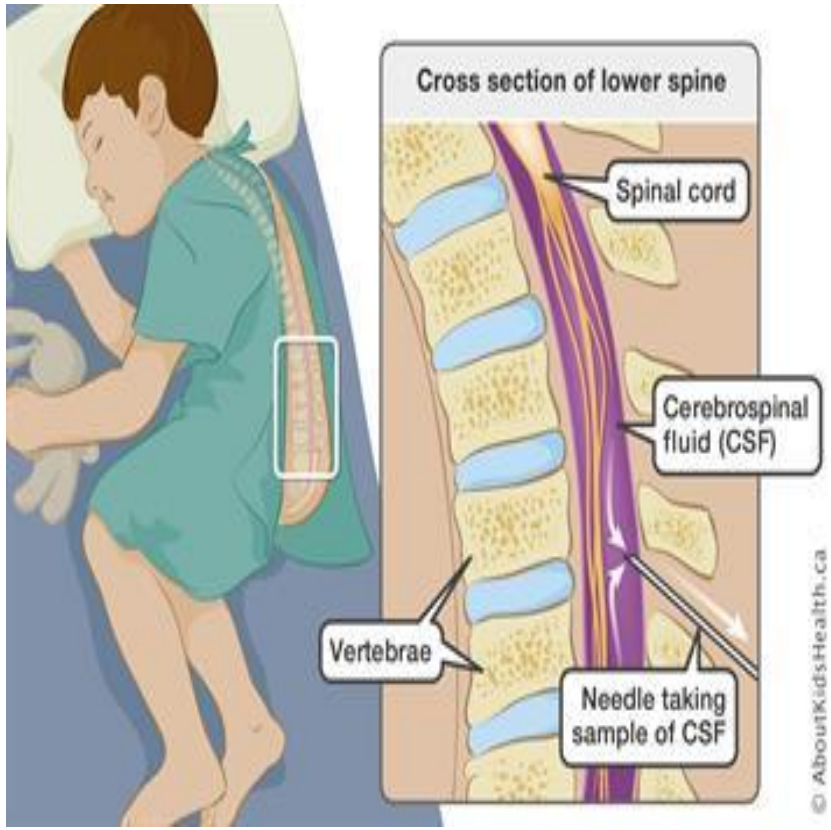
1. **Meningitis**
   - *Cronobacter* get into the blood and make the lining of the brain and spine swell (**meningitis**)
   - Symptoms: Fever, poor feeding, grunting respirations, crying, very weak, some may have seizures
   - Babies with meningitis may develop serious and long-lasting problems in their brains.
   - 4 out of 10 babies with meningitis may die.



Meningitis is an **inflammation** (swelling) of the protective membranes covering the **brain and spinal cord**.

The swelling is caused by **bacterial infection** in the **fluid** surrounding the **brain** and **spinal cord.**





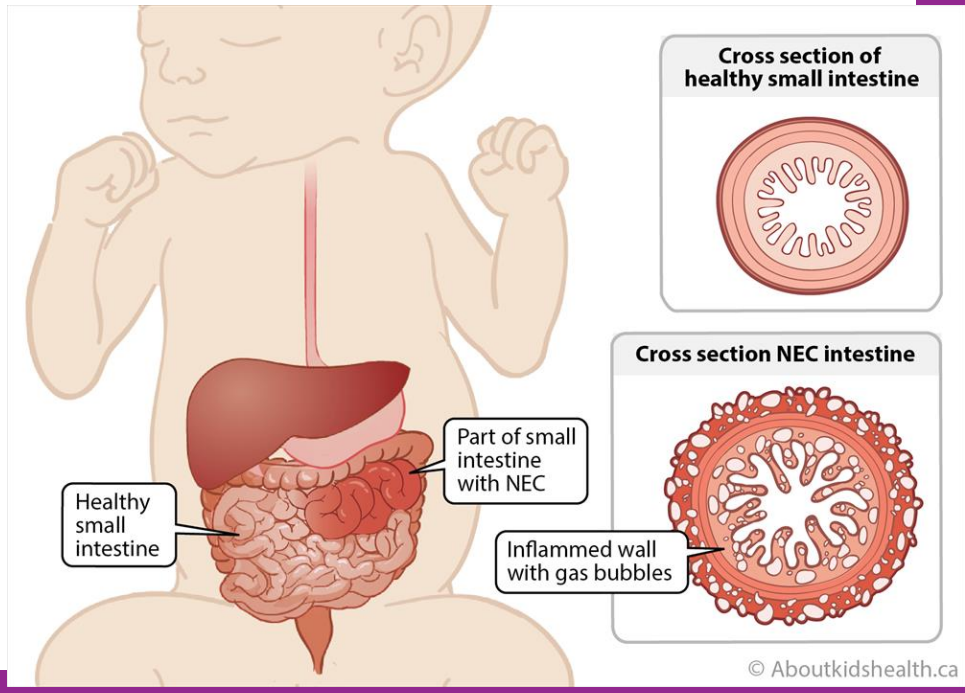
Diseases caused by *Cronobacter* infections

- Babies (less than 2 months)

2. Necrotizing enterocolitis (NEC)

- Medical condition where a portion of the small or large intestines of newborn infants die due to the infections
- Factors that increase NEC: prematurity, formula feeding, difficult birth delivery,
- Symptoms: Poor feeding, bloating, vomiting, bloody stool
- Treatment: antibiotic or removal of the affected area

Normal (top) versus necrotic section of



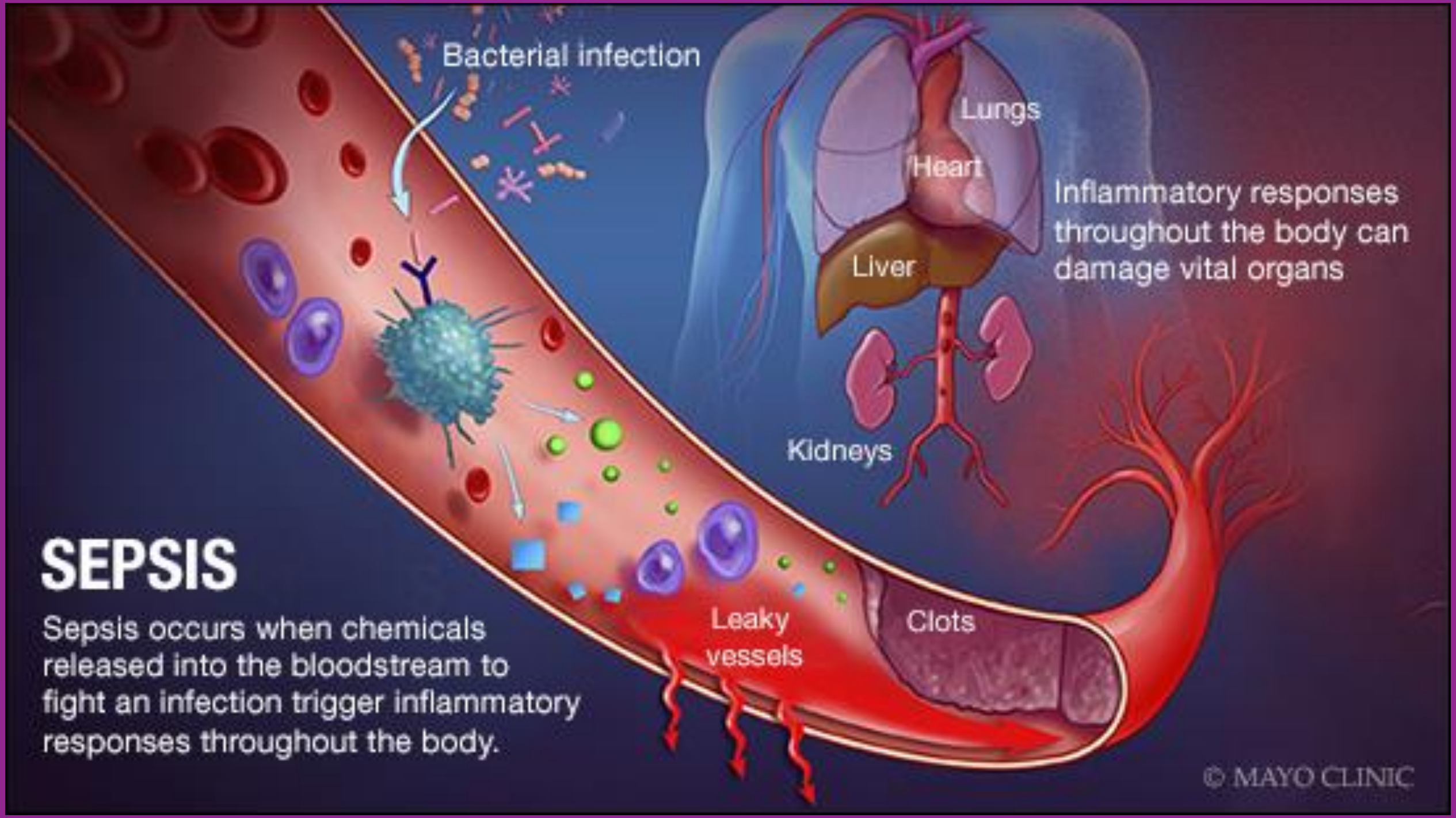
- Adults

  - Usually no health complication in healthy adults
  - Problems in **elderly** and **immunocompromised individual** (cancer patients, HIV patients, organ transplants are more susceptible) - bacteraemia
  - May cause diarrhea and urinary tract infections
  - Problems in cuts, scrapes, or places where people have had operations

  - Diagnosis: blood sampling, testing of cerebrospinal fluid for leukocytes count

Bacteria in bloodstream

- **Bacteraemia** – Presence of bacteria in the bloodstream, less amount of bacteria, may result from wound infection, surgical procedure etc.., no symptoms/mild fever, can be rapidly removed from blood stream by immune system
- **Septicaemia** – Large amount of bacteria presence and actively multiply in the bloodstream, life threatening infection, symptoms: fever, chills, fast respiration, increase heart rate and quickly leads to sepsis if untreated. Antibiotic is needed.
- **Sepsis** – body's extreme response to the bacterial infection in bloodstream that can lead to tissue damage, organ failure and death. 3 stages; sepsis, severe sepsis, septic shock. Sepsis happens when an infection you already have —in your skin, lungs, urinary tract, or somewhere else—triggers a chain reaction throughout your body (high heart rate, fever, shivering, or feeling cold, shortness of breath, extreme pain, confusion)
- **Septic shock** – severe condition that occurs when the blood pressure falls and organ shut down
- **Meningitis** – when infection reaches the lining around the brain and spinal cord (the meninges) which can cause inflammation


Bacterial infection
Lungs
Heart
Liver
Kidneys
Inflammatory responses throughout the body can damage vital organs
SEPSIS
Sepsis occurs when chemicals released into the bloodstream to fight an infection trigger inflammatory responses throughout the body.
Leaky vessels
Clots
© MAYO CLINIC

Outbreak

- 17 neonates were infected *with Cronobacter sakazakii* in a neonatal intensive care unit (NICU) – France
- The infections caused Necrotizing Enterocolitis (NEC), septicaemia, & meningitis
- 3 death

https://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-8-64

Danone Dumex formula recalled in Singapore after cronobacter sakazakii bacteria detected

By Gary Scattergood
21-Aug-2018 - Last updated on 05-Dec-2018 at 22:04 GMT

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Product Recall

Singapore's Agri-Food & Veterinary Authority (AVA) has recalled a batch of Dumex Mamil Gol Infant Milk Formula - Step 1 (850g) after detecting cronobacter sakazakii bacteria in samples

The product, imported by Danone Dumex, was manufactured in Malaysia. The effected batch number is 09117R1 with an expiry date of September 11 2019.

AVA said in a statement: *"Consumers who have purchased the affected product are advised not to consume it."*

Cronobacter sakazakii is a bacterium found in the environment that can survive dry conditions, such as products like infant formula and powdered milk. Cronobacter infections are generally rare, however they can be fatal to newborns as they may cause meningitis or sepsis.

AVA added: *"Examples of symptoms displayed by infants with cronobacter infection include fever, poor feeding or lethargy. As such symptoms are not specific to the bacteria, parents or caregivers should seek medical assistance should their infants feel unwell."*

https://www.healio.com/pediatrics/gastrointestinal-conditions/news/online/%7B6b989590-eb7b-4643-9db5-3cd32bc915b9%7D/contaminated-breast-pump-linked-to-cronobacter-infection-in-preterm-infant

Contaminated breast milk pump leaves preterm infant severely ill

Cleaning best practices highlighted after rare infection

CBC News · Posted: Jul 20, 2017 4:02 PM ET | Last Updated: July 21, 2017

Once pumped, breast milk needs to be stored carefully, health officials in the U.S. warn after a preemie developed meningitis by contaminated milk from a breast pump. (Shutterstock)

A preterm infant developed severe meningitis and was left with destroyed brain tissue after being fed milk from a contaminated breast pump, say U.S. public health officials.

The Pennsylvania girl was born prematurely, at around 29 weeks. Doctors consider a baby preterm if born before 37 weeks of pregnancy.

- No powdered formulas were used.
- *C. sakazakii* was found in the valves of the breast pump kit on 7 days before onset, 11 frozen milk samples and the drain of the kitchen sink.
- "The infant developed signs of sepsis at age 21 days. Despite treatment with ampicillin and cefepime, she developed seizures."
- The infant had also developed spastic cerebral palsy and dependent on VP shunt (operation to absorb excess cerebrospinal fluid) and a gastrostomy feeding tube.

Walmart Pulls Infant Formula After Baby's Death

Walmart pulls formula after infant's death from rare bacterial infection.

Dec. 22, 2011— -- The death of a 10-day-old Missouri infant from what early tests indicate was a bacterial infection has prompted Walmart to pull cans of infant formula from 3,000 of its stores nationwide.

The company pulled 12.5 ounce cans of Enfamil Premium Newborn formula, lot number ZP1K7G, after it learned that an infant, identified by other media outlets as Avery Cornett of Lebanon, Mo., had consumed the formula before he became sick. Preliminary tests show he developed a rare infection from Cronobacter sakazakii, a bacteria that has previously been found in powdered infant formula.

"This is not a formal government recall. We just did this out of an abundance of caution, and we're currently holding the product until the investigation is complete. The product could possibly be returned to shelves at a later date," a Walmart spokesperson told ABC News Radio.

YOUTUBE:

https://youtu.be/m47Kse6ACEQ

Enfamil Recall: Mead Johnson Says New Tests Did Not Detect Cronobacter

anikandan Raman
/11 AT 6:09 AM

Mead Johnson Nutrition Co. (NYSE: MJN) has confirmed the safety of its Enfamil infant formula saying that new tests did not detect the presence of Cronobacter (Enterobacter sakazakii) in the product.

These new results reaffirm the testing conducted before the batch was made available to retailers and consumers. Based on both sets of tests, Mead Johnson can say with confidence that Enfamil Premium Newborn formula, like every infant formula the company produces, is safe, the company said in a statement.

The Glenview, Illinois-based company added it undertook the highly unusual retesting due to

Prevention & control

- **Breastfeeding** is the better option to prevent infections
- **Proper sanitation** for feeding equipment:
  - Clean the breast pump (read the manufacturer's instruction), work surface, feeding bottles & it's accessories, feeding tube etc..
  - Method: heated water, UV radiation, chemical agents such as chlorine-based, quaternary ammonium compound sanitizers, hydrogen peroxide.
- **Wash hand** before preparing the infant formula
- Keep powdered formula lids and scoops clean (be careful about what they touch) & close the container a.s.a.p.
- Mixing the formula with water hot enough to kill pathogens (70C)
- Proper **storage** after mixing:
  - <4 hours at room temperature
  - 24 hours at 4C

THANK YOU