Food-borne pathogens

Sabina Purkrtové
Foodborne disease

Definition of foodborne disease by the World Health Organization (WHO)

‘Any disease of an infectious or toxic nature caused by, or thought to be caused by, the consumption of food or water.’

• includes all food and waterborne illness
• illnesses primarily associated with the gastrointestinal tract and exhibiting symptoms such as diarrhoea and/or vomiting, but also those present with other symptoms such as paralytic shellfish poisoning, botulism and listeriosis as well as those caused by toxic chemicals
• but excludes illnesses due to allergies and food intolerances
Foodborne disease

• most of which are of microbial origin
  – the risk of becoming ill as a result of microbial contamination of food was 100 000 times greater than the risk from pesticide contamination

• perhaps the most widespread problem in the contemporary world

• an important cause of reduced economic productivity
Foodborne diseases

Different kind of illness
• Most present as an acute gastroenteritis characterized by diarrhoea and vomiting (symptoms confined to the gut and its immediate vicinity), often described as „food poisoning“, although it can be caused by a number of different mechanisms.
• But several foodborne illnesses, such as typhoid fever, botulism and listeriosis, involve body sites remote from the alimentary tract which serves simply as the route by which the pathogen or toxin gains entry to the body.
• So the spectrum of effects on the human health is from relatively mild, self-limiting gastrointestinal upsets through to life-threatening conditions such as botulism.

Some foodborne infections can develop severe complications
• haemolytic uraemic syndrome associated with about 10 % of E. coli O157:H7 infections
• the neurological disorder Guillain-Barre’ syndrome that follows about 0.1% of Campylobacter infections.

Some groups of people are particularly susceptible
• to the more serious consequences of food borne disease
• These include the elderly, infants and those who are immunocompromised as a result of illness or chemotherapy
GASTROINTESTINAL TRACT

Function: Digestion and absorption of food and the excretion of waste

Mouth – food mixed with saliva and broken down mechanically

Saliva: alkaline fluid containing starch-degrading (amylase) enzymes and the antimicrobial immunoglobulin (IgA), lysozyme, lactoferrin and lactoperoxidase

Microflora
- depends on consumed food and the range of micro-environments
- dental plaque on the teeth— an organic biofilm in which bacteria are embedded in a matrix derived from salivary glycoproteins and microbial polysaccharides
  - Common components: filamentous *Fusobacterium* streptococci

http://www.gistsupport.org/about-gist/what-is-gist.php
GASTROINTESTINAL TRACT

Stomach
• food is blended with gastric juice, an acidic fluid containing hydrochloric acid
• proteases as pepsin and lipase
• partially digested mixture of food and gastric juice (chyme) is released into small intestine
• pH: 0.8-5.0 (usually 2.0-3.0)
  • kills most bacteria
  • normally only acid-tolerant vegetative cells and spores survive
• bacteria can evade
  • by sheltering in food particles
  • by accelerated passage through (e.g. when the stomach is full)
• when acidity is neutralized by the food
• when acidity is absent as a result of illness

Microflora in a stomach
• the microbial count is low
• lactobacilli are frequently found associated with the stomach wall
**GASTROINTESTINAL TRACT**

**Small intestine – digestion and absorption**
- 6 m of long, extensively folded
- covered with finger-like projections or villi (covered by microvilli) – nutrients and water are absorbed, passively or more often against a concentration gradient

**Duodenum** (2 %) – chyme mixed with
- digestive juice from pancreas and bile from the gallblader (neutralisation, bile salts emulsify fats)
- digestive enzymes for disaccharides and peptides from the crypts of Lieberkühn in the duodenum

**Jejunum and ileum** - food is swept by peristaltics, the flux decreased as water is absorbed

**Microflora in small intestine:**
- mainly lactobacilli and streptococci
- increasing from the duodenum (10⁵-10⁶/ml) to the jejunum (10³-10⁴/ml) and thence into the upper ileum (10⁴/ml) and the lower ileum (10⁶/ml)
- due to the flow rate in duodenum only microorganisms able to adhere can persist there
**GASTROINTESTINAL TRACT**

**Colon (large intestine)**
- active absorption of water and salts
- long periods of remaining material before expulsion - extensive microbial growth
- faeces
  - bacterial cells 25-30 % \((10^{10}-10^{11} \text{ cfu/g})\)
  - indigestible components of food
  - epithelial cells shed from the gut
  - minerals and bile

**Microflora**
- obligate anaerobes as *Bacteroides* and *Bifidobacterium* (99 %)
- *Enterobacteriaceae* (most commonly E. coli – also B\(_{12}\) production) around \(10^6 \text{ cfu/g}\)
- enterococci \(10^5 \text{ cfu/g}\)
- *Lactobacillus, Clostridium* and *Fusobacterium* \(10^3-10^5 \text{ cfu/g}\)
- other as yeasts, staphylococci and pseudomonads at lower levels
- a normal gut microflora – some protection against infection

**GUT microflora** = small + large intestine
More than 400 different species
Around \(10^{14}\) more than the number of cells in the human body

(E.g. *Clostridium difficile* is present in very low numbers, but if the balance of the flora is altered by ATB therapy, it can colonize the colon releasing toxins)
TOXINS

Two types of bacterial toxins

Lipopolysaccharides (LPS) – associated with the cell walls of Gram negative bacteria – a components of the Gram negative bacterial outer membrane = ENDOTOXINS (released after cell lysis) - pyrogenic (fever producing)

Proteins – usually released into the extracellular environment of pathogenic bacteria = most of them EXOTOXINS

(a) Exotoxins are produced inside mostly gram-positive bacteria as part of their growth and metabolism. They are then secreted or released following lysis into the surrounding medium.

(b) Endotoxins are part of the outer portion of the cell wall (lipid A; see Figure 4.12c) of gram-negative bacteria. They are liberated when the bacteria die and the cell wall breaks apart.

http://classes.midlandstech.com/carterp/Courses/bio225/chap15/15-04_Toxins_1.jpg
**BACTERIAL PROTEIN TOXINS**

Protein toxins resemble enzymes
- proteins
- denatured by heat, acid, proteolytic enzymes
- a high biological activity (most act catalytically)
- exhibit specificity of the action (in the substrate and the mode of action)
- terms as „enterotoxin“ (act on the intestinal mucosa generally causing diarrhoea), „neurotoxin“, „leukocidin“, „hemolysin“ etc. are used to indicate the target site of some well-defined protein toxins

Some protein toxins
- very specific cytotoxic activity (they attack specific cells – e.g. tetanus or botulinum toxins)
- some fairly broad cytotoxic activity and cause nonspecific death of tissues (necrosis)
- phospholipases – relatively nonspecific
- few cause death of the host – lethal toxins (e.g. anthrax toxin)

Many consist of two components:
subunit A: the enzymatic activity of toxin
subunit B: binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane
LIPOCHOLYPOLYSACCHARIDES

Gram-negative bacterial endotoxin (lipopolysaccharide, LPS)

O-specific polysaccharide chain

Core glycolipid

Lipid A

$O$-specific oligosaccharide subunit

$O$-specific oligosaccharide

(inner)

(core oligosaccharide)

http://lipidlibrary.aocs.org/Lipids/lipidA/Figure1.png

Endotoxins

- heat stable (boiling for 30 min does not destabilize endotoxin)
- less potent and less specific in their action, since they do not act enzymatically
- released from multiplying or disintegrating bacteria significantly contribute to the symptoms of Gram negative bacteremia and septicemia

**Lipid A is the toxic component**

- a very potent stimulant of the immune system
- Lipid A is known to react at the surfaces of macrophages – they release cytokines that mediate the pathophysiological response to endotoxin
- at high concentrations during a Gram-negative bacterial infection, it may cause shock and death by an "out of control" excessive immune reaction

http://www.slideshare.net/MMASSY/bacterial-toxins
BACTERIAL FOOD POISONING

Ingestion of pre-formed toxin
Toxins may be produced in and ingested with the food
Relatively short incubation period
Absence of person to person spread
- *Staphylococcus aureus* enterotoxins, *Bacillus cereus* emetic syndrome
- Botulism (but gastrointestinal symptoms are of minor importance)

Non-invasive infection
- Viable bacteria colonize the intestinal lumen by adhering onto the epithelial surface by adhesins
  - (usually the small intestine – less intense competition)
- Once attached they produce a protein enterotoxin
  - changes the flow of electrolyte and water across the mucosa (and from absorption to secretion)
  - or stimulating enterocytes to over-produce cyclic nucleotides
**BACTERIAL FOOD POISONING**

**Invasive infection**
- bacteria invade the cells, but do not normally spread much beyond the immediate vicinity of the gut
- *Salmonella* preferentially invade the ileum
  - pass through the epithelial cells to multiply in lamina propria
  - production of heat-labile enterotoxin, cytotoxin
  - the local acute inflammation (fever and chills) causes an increase in levels of prostanglandis (activators of adenylate cyclase) – watery diarrhoea

- *Shigella* and *enteroinvasive E. coli* invade the colonic mucosa
  - adhere to the enterocytes via outer membrane protein adhesins
  - engulfed by the enterocytes in response to a phagotic singal
  - multiply within the cytoplasm invading adjacent cells and the unerlying connective tissue – strong inflammatory response
  - dysenteric syndrome = inflammation, abscesses and ulceration of the colon, the passage of bloody, mucus-and pus-containing sools
  - Some produce Shiga toxin – its role in the pathogenesis remain unclear
BACTERIAL FOOD POISONING

Invasive infection

http://www.mdpi.com/2072-6651/6/2/430/htm
CHOLERA TOXIN

- produces by *Vibrio cholerae*
- MW 84 000 Da
- after ingestion *Vibrio cholerae* colonizes the small intestine and secretes cholera toxin
- five B subunits to bind to specific ganglioside (an acidic glycolipid) receptors on the enterocyte surface
- a single A subunit passes through a hydrophilic channel in the cell membrane
- the A unit acts enzymically to transfer an ADP-ribosyl group to a protein regulating the activity of the enzyme adenylate cyclase
- the adenylate cyclase is locked into its active state – an accumulation of cyclic adenosine monophosphate (cAMP)
- the absorption of Na+, Cl- is inhibited, while their secretion of Cl-, HCO$_3^-$ and Na+ is stimulated
- to maintain an osmotic balance the transfer of electrolytes is accompanied by a massive outflow of water into the intestinal lumen – exceeds the absorptive capacity of the large intestine – watery diarrhoea
Heat labile toxin (LT) produced by some types of enterotoxigenic *E. coli* – act the same way as cholera toxin
Heat stable toxin of *E. coli* similar to stimulate the production of cGMP (cyclic guanosine monophosphate), which differs slightly from cAMP but also produces diarrhoea as a result of electrolyte imbalances

**CLOSTRIDIUM PERFRINGENS ENTEROTOXINS**

Released upon sporulation of the organism in the small intestine.
Its proposed mode of action involves
(1) activation
(2) binding to cellular receptors
(3) creating perforations in intestinal epithelial cells through a series of reactions, thus increasing permeability of the membrane to small macromolecules (<200 Daltons in size)
Shiga toxins are a family of related toxins with two major groups, Stx1 and Stx2, expressed by genes considered to be part of the genome of lambdoid prophages. The toxins are named for Kiyoshi Shiga, who first described the bacterial origin of dysentery caused by *Shigella dysenteriae*. The most common sources for *Shiga toxin* are the bacteria *S. dysenteriae* and the shigatoxigenic group of *Escherichia coli* (STEC), which includes serotypes O157:H7, O104:H4, and other enterohemorrhagic *E. coli* (EHEC).
• **a type III secretion system** is used for secretion of proteins involved in the signalling events, which subvert the host cell and lead to bacterial uptake receptor mediated endocytosis.

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**Endocytosis**

- **Phagocytosis**
  - Solid particle
  - Plasma membrane
  - Pseudopodium
  - Phagosome (food vacuole)

- **Pinocytosis**
  - Extracellular fluid
  - Vesicle
  - Cytoplasm

- **Receptor-mediated endocytosis**
  - Coated pit
  - Receptor
  - Coat protein
  - Coated vesicle
### Table 6.2 Some microbiological agents of foodborne illness

<table>
<thead>
<tr>
<th>Agents</th>
<th>Important reservoir/carryer</th>
<th>Transmission&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Multiplication in food</th>
<th>Examples of some incriminated foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BACTERIA:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aeromonas</em></td>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>Cooked rice, cooked meats</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Soil</td>
<td>−</td>
<td>+</td>
<td>Vegetables, starchy puddings</td>
</tr>
<tr>
<td><em>Brucella species</em></td>
<td>Cattle, goats, sheep</td>
<td>−</td>
<td>+</td>
<td>Raw milk, dairy products</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Chickens, dogs, cats, cattle, pigs, wild birds</td>
<td>+</td>
<td>+</td>
<td>Raw milk, poultry</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em></td>
<td>Soil, mammals, birds, fish</td>
<td>−</td>
<td>+</td>
<td>Fish, meat, vegetables (home preserved)</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Soil, animals, man</td>
<td>−</td>
<td>+</td>
<td>Cooked meat and poultry, gravy, beans</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterotoxigenic</td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>Salads, raw vegetables</td>
</tr>
<tr>
<td>Enteropathogenic</td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>Milk</td>
</tr>
<tr>
<td>Enteroinvasive</td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>Cheese</td>
</tr>
<tr>
<td>Entero-haemorrhagic</td>
<td>Cattle, poultry, sheep</td>
<td>+</td>
<td>+</td>
<td>Undercooked meat, raw milk, cheese</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td></td>
<td>−</td>
<td>+</td>
<td>Soft cheeses, milk, coleslaw, pate</td>
</tr>
<tr>
<td><em>Mycobacterium bovis</em></td>
<td>Cattle</td>
<td>−</td>
<td>+</td>
<td>Raw milk</td>
</tr>
<tr>
<td><em>Salmonella Typhi</em></td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>Dairy produce, meat products, shellfish, vegetable salads</td>
</tr>
<tr>
<td><em>Salmonella</em> (non-Typhi)</td>
<td>Man and animals</td>
<td>±</td>
<td>+</td>
<td>Meats, poultry, eggs, dairy produce, chocolate</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>Potato/egg salads</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (enterotoxins)</td>
<td>Man</td>
<td>−</td>
<td>+</td>
<td>Ham, poultry and egg salads, cream-filled bakery produce, ice-cream, cheese</td>
</tr>
<tr>
<td><em>Vibrio cholerae O1</em></td>
<td>Man, marine life?</td>
<td>+</td>
<td>+</td>
<td>Salad, shellfish</td>
</tr>
<tr>
<td><em>Vibrio cholerae, non-O1</em></td>
<td>Man and animals, marine life?</td>
<td>+</td>
<td>+</td>
<td>Shellfish</td>
</tr>
</tbody>
</table>
## Table 6.2 (continued)

<table>
<thead>
<tr>
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<th>Examples of some incriminated foods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>water</td>
<td>food</td>
<td>person to person</td>
</tr>
<tr>
<td><em>Vibrio para-haemolyticus</em></td>
<td>Seawater, marine life</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Water, wild animals, pigs, dogs, poultry</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>VIRUSES:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Norovirus</td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Man</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>PROTOZOA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em></td>
<td>Man, animals</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>Man, animals</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>HELMINTHS:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>Man</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Taenia saginata</em> and <em>T.</em></td>
<td>Cattle, swine</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>solium</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichinella spiralis</em></td>
<td>Swine, carnivora</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>Man</td>
<td>0</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Almost all acute enteric infections show increased transmission during the summer and/or wet months, except infections due to rotavirus and *Yersinia enterocolitica*, which show increased transmission in cooler months.

<sup>b</sup> Under certain circumstances some multiplication has been observed. The epidemiological significance of this observation is not clear.

- + = Yes
- ± = Rare
- - = No
- 0 = No information

Adapted from WHO 1992

Adams and Moss, 2008
Foodborne diseases

Figure 1. Reported notification rates of zoonoses in confirmed human cases\(^{(b),(c)}\) in the EU, 2013

Foodborne diseases

Bacterial toxins include toxins produced by *Bacillus, Clostridium* and *Staphylococcus*. Food-borne viruses include calicivirus, hepatitis A virus, flavivirus, rotavirus and other unspecified viruses. Other causative agents include mushroom toxins, marine biotoxins, histamine, mycotoxins and escolar fish (wax esters). Parasites include primarily *Trichinella*, but also *Cryptosporidium, Giardia* and other unspecified parasites. Other bacterial agents include *Listeria, Brucella, Shigella, Vibrio* and other unspecified bacterial agents. In this figure, the category ‘Pathogenic *Escherichia coli* (including VTEC)’ also includes one strong-evidence outbreak due to pathogenic *E. coli* other than VTEC.

*Figure 2. Distribution of all food-borne outbreaks per causative agent in the EU, 2013*

Enterobacteriaceae and coliform bacteria

Family Enterobacteriaceae
found in soil, water, plants and animals and their gastrointestinal tracts
Gram-negative, straight rods, some of which motile
Most grow well at 37 °C, some better at 25 - 30°C,
facultatively anaerobic, fermenting glucose,
oxidase-negative and catalase-positive (except Shigella dysenteriae type 1),
resistant to bile salts
Salmonella sp., Shigella sp., Yersinia sp., Cronobacter sp.
Proteus sp., Hafnia sp., Pantoea sp. etc.

coliform bacteria = subgroup of Enterobacteriaceae
similar to E. coli = coliform
may be found also as normal intestinal flora of humans and/or animals, considered opportunistic pathogens - indicator for faecal contamination in water or bad hygiene/insufficient storage conditions in food
Escherichia sp., esp. Escherichia coli; Enterobacter sp., Klebsiella sp., Citrobacter sp.
fermenting lactose (enzyme β-D-galactosidase)

Escherichia coli β-D-glucuronidase (app. 97 % of strains), indole positive
Enterobacteriaceae and coliform bacteria

Biochemical properties used for isolation on selective media and confirmation

Family Enterobacteriaceae
Gram-negative, resistant to bile salts
fermenting glucose, oxidase-negative,
cultivation temperature: 37 °C

coliform bacteria
all features of Enterobacteriaceae
+ fermenting lactose (enzyme β-D-galactosidase)

Escherichia coli
all features of Enterobacteriaceae and coliforms
+ enzyme β-D-glucuronidase
(app. 97 % of strains, not present e.g. in E. coli O157)
indole positive
Salmonella spp.

- **Family Enterobacteriaceae**
  - Gram-negative rod non-sporeforming rods (typically 0.5 µm by 1–3 µm)
  - facultatively anaerobic
  - catalase positive, oxidase-negative
  - generally motile with peritrichous flagella
- **growth temperature**: 5-47 °C, an optimum 37 °C
- **heat sensitive, but more resistant in low** $a_w$
  - in milk:
    - $D_{72} = 0.09$ min for the most heat resistant *S. Senftenberg 775W*
    - $D_{72} = 0.003$ min for *S. Typhimurium*
    - but in chocolate sauce
      - $D_{70} = 11.3–17.5$ h for *S. Typhimurium*
- **growth pH**: optimum around pH 7
  - the minimum growth pH from 5.4 (acetic acid) to 4.05 (hydrochloric and citric acids)
Salmonella spp.

The genus Salmonella should currently comprise two species
- *Salmonella enterica* (divided into six subspecies)
- *Salmonella bongori* (previously belonged to subgenus V)

Different serovars (more than 2500) is characterised by the antigenic structure = present types of O, H and Vi antigens

![Antigenic structure diagram]

antigenic formulae

(i) O antigens letters and numbers, 69 types:
   separated by a comma
(ii) phase 1 H antigens (a-z):
    separated by a colon
(iii) phase 2, if present (1-12)

*Salmonella enterica* subsp. *enterica* serovar Typhimurium = *Salmonella* Typhimurium
(4,5,12:i:1,2)

White–Kauffmann–Le Minor scheme

- **O** (somatic) antigens
  - lipopolysaccharides
- **H** (flagellar) antigens
  - protein (flagellin)
  - phase 1 H antigens and phase 2
    - different in the primary structure
    - different genes, expressed alternantly
- **Vi** (capsular) antigen
  - polysaccharide
  - present only in some serovars
    (e.g. S. Typhi, S. Dublin a S. Hirschfeldii)
Salmonella spp.

In subspecies I (S. enterica subsp. enterica) serovars are designated by a name (usually indicative of associated diseases, geographic origins, or usual habitats)

<p>| Table 1 Current Salmonella nomenclature (adapted from Lin-Hui and Cheng-Hsun 2007) |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>Taxonomic position (writing format) and nomenclature</th>
<th>Number of serovars</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus (capitalised, italic)</td>
<td>Species (not capitalised, italic)</td>
<td>Subspecies (symbol) (not capitalised, italic)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>enterica</td>
<td>enterica (subspecies I)</td>
</tr>
<tr>
<td></td>
<td>salamae (subspecies II)</td>
<td>9,46:z:z39</td>
</tr>
<tr>
<td></td>
<td>arizonae (subspecies IIIa)</td>
<td>43:z:29:-</td>
</tr>
<tr>
<td></td>
<td>diarizonae (subspecies IIIb)</td>
<td>6,7:l:v:1,5,7</td>
</tr>
<tr>
<td></td>
<td>houtenae (subspecies IV)</td>
<td>21:m:t:-</td>
</tr>
<tr>
<td></td>
<td>indica (subspecies VI)</td>
<td>59:z:36:-</td>
</tr>
<tr>
<td></td>
<td>(Subspecies V)</td>
<td>13,22:z:39:-</td>
</tr>
<tr>
<td>Salmonella</td>
<td>bongori</td>
<td></td>
</tr>
</tbody>
</table>

Agbaje et al. (2011)

Primarily inhabitants of the gastrointestinal tract
• a wide host range, usually without the display of any apparent illness
  • food animals, wild animals, rodents, pets, birds, reptiles, and insects...
• the host adaptability - classification into three groups:
• **Group 1**: adapted to man and higher primates, e.g. Salmonella Typhi, Paratyphi A, B, C and Sendai
• **Group 2**: wholly or largely adapted to specific animals, e.g. S. Dublin in cattle, S. Gallinarum in poultry, S. Abortusequi in horses, S. Choleraesuis in pigs but infrequently in humans
• **Group 3**: all other that are not host adapted and produce infection in man and a wide range of animals, e.g. S. Typhimurium and S. Enteritidis
**Salmonella spp.**

**Enteritis**
- gastrointestinal infection
- the most common type of salmonellosis (often called directly as „salmonellosis“)
- severity: from asymptomatic carriage to severe diarrhoea
- predominantly associated with those serovars which occur widely in animals and humans

- the most common serovars for enteritis (non-typhoidal) infection: **S. Enteritidis, S. Typhimurium** (other: S. Virchow, S. Infantis, S. Newport...)

![Distribution of the 10 most common Salmonella serovars in humans in the European Union, 2012 (N=82,409)](http://www.eurosurveillance.org/images/dynamic/EE/V19N19/Hugas_fig1.jpg)
Salmonella spp.

Enteritis

- adhesion to the epithelial cells of the ileum via mannose-resistant fimbriae
- a **type III secretion system** is used for secretion of proteins involved in the signalling events, which subvert the host cell and lead to bacterial uptake receptor mediated endocytosis
- endocytosed salmonellas pass through the epithelial cells within a membrane-bound vacuole, where they multiply and are then released into the lamina propria
- an influx of inflammatory cells leads to the release of prostaglandins which activate adenylate cyclase producing fluid secretion into the intestinal lumen

Salmonella infection

Almost any kind of food or beverage can carry the bacteria that causes salmonella infection, although meat and eggs the most are common sources.

**Symptoms**
- Within 12-72 hours: Nausea, vomiting, fever, diarrhea abdominal cramps
- **4-7 days**: Illness ranges from mild to severe; most people recover without treatment
- **Severe cases**: More likely with infants, elderly, people with impaired immune systems

**Treatment**
- Oral or injected antibiotics, usually for 2 weeks

Source: U.S. Food and Drug Administration, Current Medical Diagnosis & Treatment, Mayo Clinic

McClatchy-Tribune
Salmonella spp.

**Typhoid and paratyphoid fever**
- Systemic disease caused by human-adapted serovars (*typhoidal salmonella*)
- *S. Typhi*, and *S. Paratyphi A, B, and C*
- Human carriers are the only source of infection
  - Connected with water supply, poor sanitary conditions, contaminated food
  - Mainly in the developing countries
- From mild to severe symptoms, but **without prompt treatment (ATB), it can cause serious complications and can be fatal (mortal rate up to 25%)**
- Vaccination
- **Host-adapted serotypes are more invasive and tend to systemic disease as to be resistant to phagocytic killing**

[Diagram of bacterial infection and spread](http://www.atsu.edu/faculty/chamberlain/website/lectures/tritzid/shigello.htm)
Salmonella spp.

**Enteritis** = a zoonotic infection
- the major source of human illness is infected animals
- transmission: the faecal–oral route
- contamination of food or water by intestinal contents from an infected animal
  - temperatures allows the salmonellae to grow in the food
  - inadequate or absent final heat treatment (undercooked)
  - cross-contamination of other foods (direct contact or indirectly via equipment and utensils)
- direct person-to-person spread is usually restricted to institutional outbreaks (hospitals, old people’s homes, and nurseries)

Salmonella spp.

Enteritis (salmonellosis) - Primary vehicles
- meat, milk, poultry and eggs
  - raw or uncooked, cross-contamination
- salad vegetables, fruits (e.g. melone)
  - use of polluted irrigation water or human and animal manure as fertilizer
- nuts, spices, seed...

*These contaminated ingredients or single foods (belonging to one food category) were associated with 1/3 of the Salmonella outbreaks.
†Other includes: Sprouts, leafy greens, roots, fish, grains-beans, shellfish, oil-sugar, and dairy.

Escherichia coli

- Family *Enterobacteriaceae*
  - Gram-negative rod non-sporeforming rods (typically 0.5 µm by 1–3 µm)
  - facultatively anaerobic
  - catalase positive, oxidase-negative
  - generally motile with peritrichous flagella
- growth temperature: 7-10 to 50 °C, an optimum 37 °C
- heat sensitive
  - $D_{60} = 0.1$ min
- growth pH: optimum around pH 7
  - the minimum growth pH 4.4 (under otherwise optimal conditions)
- growth $a_w$: minimum 0.95

http://www.ecl-lab.ca/en/ecoli/

Universal inhabitant of the gut of humans and other warm-blooded animals
- the predominant facultative anaerobe, but only a minor component of the total microflora
- generally a harmless commensal
- an opportunistic pathogen - infections such as Gram-negative sepsis, urinary tract infections, pneumonia in immunosuppressed patients, meningitis in neonates
- only a small proportion of E. coli strains are pathogenic
  - possessing and producing virulence factors permitting them to cause disease

The uptake of mobile genetic elements (phages, virulence plasmids and pathogenicity islands), as well as the loss of chromosomal-DNA regions in different E. coli lineages, has enabled the evolution of separate clones, which belong to different E. coli pathotypes and are associated with specific disease symptoms.
LEE, locus of enterocyte effacement; PAI, pathogenicity island; pEAF, enteropathogenic E. coli adhesion-factor plasmid; pENT, enterotoxin-encoding plasmids; Stx, Shiga-toxin-encoding bacteriophage
Escherichia coli

Diarrheagenic Escherichia coli — pathogenic E. coli — based on virulence markers*

- ETEC: Enterotoxigenic E. coli
- EPEC: Enteropathogenic E. coli (EAF and EAE)
- EHEC: Enterohemorrhagic E. coli (Stx and EAE) [hemorrhagic colitis (HC)-associated]
- EIEC: Entero invasive E. coli
- EAEC: Enterocolitogenus E. coli
- DAEC: Diffusely adherent E. coli

STEC: Stx-producing E. coli are STEC (many serotypes, ~ 200)

EHEC: Clinically, HC-associated pathogenic STEC are classified as EHEC

- EPEC 10^4 IHD
- ETEC 10^7 IHD

**An unusual STEC, E. coli 104:H4 demonstrates blended virulence profiles of enteric pathogens, combining both STEC and EAEC virulence characteristics within a single organism.

http://www.foodsafetymagazine.com/magazine-archive1/april-may-2012/understanding-stec-a-growing-concern/

http://www.bats.ch/bats/publikationen/1996-1_e.coli/96-1_e.coli_k12.php
Table 1. Categories of diarrheagenic *Escherichia coli*

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Category</th>
<th>Mechanism of pathogenicity</th>
<th>Major pathogenic factor or marker</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHEC/VTEC</td>
<td>Detection of Verotoxin (VT) or VT-coding gene (including detection from carriers)</td>
<td>EHEC/VTEC</td>
<td>Toxin</td>
<td>VT1, VT2</td>
<td>Unrevised</td>
</tr>
<tr>
<td>ETEC</td>
<td>Detection of heat-labile enterotoxin (LT) and/or heat-stable enterotoxin (ST) or LT/ST-coding gene</td>
<td>ETEC</td>
<td>Toxin</td>
<td>LT, ST</td>
<td>Unrevised</td>
</tr>
<tr>
<td>EIEC</td>
<td>Detection of invasion plasmid or genes related to invasion</td>
<td>EIEC</td>
<td>Invasiveness</td>
<td><em>invE, ipaH</em></td>
<td>Unrevised</td>
</tr>
<tr>
<td>EPEC</td>
<td>Classified as so called 'enteropathogenic serotypes' excluding EIEC *O group: 1; 13; 20; 56; 44; 55; 86; 111; 114; 118; 125; 136; 127; 138; 142; 146; 161; 153; 159 *excluding LT/ST/VT-positive</td>
<td>EPEC</td>
<td>Localized adherence</td>
<td><em>ese, bfpA, EAF</em></td>
<td>Localized adherence to cultured cells or detection of related genes *excluding LT/ST/VT-positive and invasiveness positive</td>
</tr>
<tr>
<td>EAgyEC</td>
<td>Aggregative adherence</td>
<td>EAgyEC</td>
<td>Aggregative adherence</td>
<td><em>aggR, CVD452</em></td>
<td>Aggregative adherence to cultured cells or detection of related genes *excluding LT/ST/VT-positive and invasiveness positive</td>
</tr>
<tr>
<td>Others</td>
<td>Not categorised by above four definitions but considered as pathogen of gastroenteritis Not detected or not tested for invasiveness and LT/ST/VT <em>E. coli</em> strains isolated from many patients involving an outbreak and characterized as same in biochemistry and classified for <em>O</em> group other than above EPEC <em>O</em> groups or <em>O</em> group-unknown</td>
<td>Others</td>
<td>Unknown</td>
<td><em>sfa, estA, CDT, cnf</em></td>
<td>Not categorised by above five definitions but considered as pathogen of gastroenteritis <em>E. coli</em> strains isolated from many patients involving an outbreak and characterized as same in biochemistry</td>
</tr>
</tbody>
</table>

EHEC: Enterohemorrhagic *E. coli*, VTEC: Verotoxin-producing *E. coli*, ETEC: Enterotoxigenic *E. coli*, EIEC: Enteroinvasive *E. coli*, EPEC: Enteropathogenic *E. coli*, EAgyEC: Entercaggregative *E. coli*

http://idsc.nih.go.jp/iasr/33/383/graph/t3831.gif
Escherichia coli

Enterohemorrhagic bacteria Escherichia coli (EHEC)

Most Escherichia coli (E. coli) strains are harmless. But some, like enterohemorrhagic E. coli (EHEC), are a hazard to human health and life.

Incubation period: three to eight days

E. coli (EHEC), once in the human stomach, begins producing toxins that cause serious illnesses.

Symptoms caused by E. coli (EHEC):
- Stomach muscle spasms
- Diarrhea (sometimes bloody diarrhea)
- Fever
- Vomiting

Complications:
hemolytic uremic syndrome (HUS)

Death rate: 3-5%

INFECTION SOURCES

Cattle and other ruminants are the main E. coli (EHEC) carriers

Uncooked meat and raw milk

The bacteria die when food is exposed to heat (70°C and higher)

Fruit and vegetables (droppings of sick animals find their way into water bodies that in turn feed the soil)

Escherichia coli

Outbreaks of EHEC serotype O157:H7
- relatively low infectious dose (2-2000 cells)
- mostly undercooked ground meat products
- occasionally raw milk
- other: lettuce, raddish sprouts, alfalfa sprouts, unpasteurised apple juice

The important reservoir of O157:H7
- cattle, pork, poultry, lamb
- e.g. in U.K. isolated from
  - 0.9–8.2% of healthy cattle
  - 3.7% from 164 samples of retailed fresh beef
  - 1–2% of other fresh meat products such as pork, poultry and lamb

http://www.ecl-lab.ca/en/ecoli/
**Escherichia coli**

An outbreak of exceptionally virulent Shiga toxin (Stx)-producing *E. coli* O104:H4 centered in Germany caused over 830 cases of hemolytic uremic syndrome (HUS) and 46 deaths since May 2011.

Serotype O104:H4, which has not been detected in animals, has rarely been associated with HUS in the past. The HUS-associated strains both carried genes typically found in two types of pathogenic *E. coli*, enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC).

The model was proposed in which EAEC 55989 and EHEC O104:H4 strains evolved from a common EHEC O104:H4 progenitor. It was suggested that by stepwise gain and loss of chromosomal and plasmid-encoded virulence factors, a highly pathogenic hybrid of EAEC and EHEC emerged as this outbreak clone.

Sprouts were finally identified as the most likely outbreak vehicle (the beginning suspicion for Spanish cucumbers was denied).
Shigella spp.

- Family Enterobacteriaceae
- Studies of DNA relatedness with *Escherichia coli* – *Shigella* spp. in fact belong to the same genus, although *Shigella* is relatively inactive biochemically when compared with *Escherichia coli*
- But because most strains of *Shigella* are pathogenic (unlike *Escherichia*) and a redesignation might cause confusion with potentially serious consequences the separate genera are retained however.
- Four species, all causing bacillary dysentery in human and higher primates with different severity of the illness
  - *Sh. dysenteriae* – severe (mainly tropical countries, rarely in Europe and North America)
  - *Sh. sonnei* – mildest (more common in Europe and North America)
  - *Sh. boydii* and *Sh. flexneri* – intermediate severity

### Shigellosis (bacillary dysentery)

- Low infectious dose (10–100 organisms)
- Incubation period: 7 h - 7 days
  - foodborne outbreaks - shorter incubation periods of up to 36 h
- Symptoms:
  - diarrhoea
    - a classic dysenteric syndrome of bloody stools containing mucus and pus (*Sh. dysenteriae*, *Sh. flexneri* and *Sh. boydii*)
    - a watery diarrhoea (*Sh. sonnei*)
  - abdominal pain, vomiting, fever
- Lasting: 3 - 14 days
  - possible to develop a carrier state, which can persist for several months
- Treatment:
  - Milder forms - self-limiting, no treatment
  - *Sh. dysenteriae* infections often require fluid and electrolyte replacement and antibiotic therapy.
**Shigella spp.**

**Shigellosis**

- the causative organism is frequently found in water polluted with human feces, and is transmitted via the fecal-oral route.
- the usual mode of transmission is directly person-to-person hand-to-mouth, in the setting of poor hygiene among children.
- **Foodborne cases**
  - not very significant
  - usually a human carrier involved in preparation of the food
  - rarely primary crop: e.g. iceberg lettuce
  - insect as the carrier
**Shigella spp.**

Shigellosis is an invasive infection where the organism’s invasive property is encoded on a large plasmid.

http://textbookofbacteriology.net/Shigella_3.html

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein Product MW</th>
<th>Regulatory or effector function</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>virF</em></td>
<td>30 kDa</td>
<td>positive regulators of the <em>virG</em> and <em>ipa-mxi-spa</em> loci</td>
</tr>
<tr>
<td><em>invA(mxiB)</em></td>
<td>38 kDa</td>
<td>Necessary for invasion (orients <em>ipa</em> gene products in outer membrane)</td>
</tr>
<tr>
<td><em>mxA</em></td>
<td>76 kDa</td>
<td>Same as above</td>
</tr>
<tr>
<td><em>ippI</em></td>
<td>18 kDa</td>
<td>Same as above</td>
</tr>
<tr>
<td><em>ipaB</em></td>
<td>62 kDa</td>
<td>Necessary for invasion: mediates endocytic uptake of shigellae</td>
</tr>
<tr>
<td><em>ipaC</em></td>
<td>43 kDa</td>
<td>Same as above</td>
</tr>
<tr>
<td><em>ipaA</em></td>
<td>38 kDa</td>
<td>Same as above</td>
</tr>
<tr>
<td><em>ipaD</em></td>
<td>78 kDa</td>
<td>Not necessary for invasion (role unknown)</td>
</tr>
<tr>
<td><em>virB</em></td>
<td>33 kDa</td>
<td>positive regulator of the <em>virG</em> and <em>ipa-mxi-spa</em> loci</td>
</tr>
<tr>
<td><em>virG</em></td>
<td>120 kDa</td>
<td>assembles actin tails that propel the bacteria through the cell cytoplasm and into adjacent cells</td>
</tr>
<tr>
<td><em>ipaH</em></td>
<td>60 kDa</td>
<td>has 5 alleles; IpaH7.8 facilitates the escape of <em>Shigella</em> from phagocytic vacuoles</td>
</tr>
<tr>
<td><em>shET2</em></td>
<td>60kDa</td>
<td>ShET2 enterotoxin</td>
</tr>
</tbody>
</table>
Yersinia enterocolitica

**Occurrence**
- widespread in the environment
- isolated from soil, fresh water, intestinal tract of many animals...
- Pigs - chronic carriers
- Food: Contaminated milk, water and cross-contamination to beansprouts

**Pathogenesis of genus Yersinia**
- *Y. pseudotuberculosis* – mesenteric adenitis
- *Y. pestis* – bubonic plague
- *Y. enterocolitica* – gastroenteritis
- Most food isolates are non-pathogenic
- A self-limiting enterocolitis mainly in children under 7 years
- An incubation time: 1-11 days
- Lasting 5-14 days
- Abdominal pain, diarrhoea, mild fever, vomiting is rare
- Possible post-infection complications: arthiris, erythema nodosum
- Produce heat-stable enterotoxin (similar to *E. coli* ST in some features)

**Family Enterobacteriaceae**
- Gram-negative rod non-sporeforming rods (typically 0.5 µm by 1–2 µm)
- facultatively anaerobic
- catalase positive, oxidase-negative
- motile with peritrichous flagella below 30 °C
- growth temperature: 1-40 °C, the optimum 29 °C
- Heat sensitive – slightly higher resistance
- $D_{62.8}$ in whole milk = 0.7-57.6 s
- Growth ph 7-8,
  - Minimum 4.1-5.1 due to used acidulant
- Possible to grow at 5 % of salt

**Heat sensitive**
- Slightly higher resistance
- $D_{62.8}$ in whole milk = 0.7-57.6 s

**Growth temperature**
- 1-40 °C, the optimum 29 °C

**Ph**
- 7-8
  - Minimum 4.1-5.1 due to used acidulant
Cronobacter spp.

- Family Enterobacteriaceae
  - Gram-negative rod non-sporeforming rods (typically 0.5 µm by 1–3 µm)
  - facultatively anaerobic
  - catalase positive, oxidase-negative
  - generally motile with peritrichous flagella
- growth temperature: 6-47 °C
- Heat sensitive – slightly higher resistance
  - $D_{60} = 2.5$ min
- More resistant to low aw stress = significant factor in its transmission

Taxonomy:
- originally - a strain of Enterobacter cloacae with the production of a yellow water diffusible pigment on tryptone soy agar
- 1980 - renamed Enterobacter sakazakii in honour of the eminent Japanese bacterial taxonomist Riichi Sakazaki
- 2008 – a new genus Cronobacter spp. (Cronus devouring his children – greek mythology)
**Cronobacter spp.**

**Pathogenesis**
- **Rare, but severe infections in infants**
  - meningitis, cerebritis, bacteraemia and necrotising enterocolitis
  - a high mortality rate of 50% or more
  - severe long term, irreversible sequelae occur in most survivors (quadriplegia, impaired sight or hearing)
  - the most common predisposing factors
    - low birthweight or premature birth
  - in people of all ages:
    - wound infections or urinary tract infections, possible bloodstream infection in the immunocompromising and the elderly

**Occurrence**
- widespread in the environment
- isolated from water, soil, vegetation, the contents of household vacuum cleaners, infant formula, skimmed milk powder, herbal teas, and starches...

**Common cause of infection : Powdered infant formula foods**
- mainly caused by post pasteurisation contamination
- **risk factors in preparation:**
  - poor hygienic practices during reconstitution
  - storage of the reconstituted product allowing bacterial multiplication

http://www.redorbit.com/media/uploads/2012/01/health-010212-003-617x416.jpg
Campylobacter spp.

- **Family** *Campylobacteraceae*
- **Genus** *Campylobacter* - 23 species
- Gram negative spirale or curved non-spores forming rods (0,2-0,8 μm × 0,5-5 μm)
  - in older cultures it can pass in cocoid forms (enzymatic degradation of the peptidoglycane layer)
- **Most species motile due to the polar flagellae**
  - uni- or bipolar, possible multipolar
  - characteristic corkscrew movement
- Mikroaerofilic bacteria (5 % O₂ + 10 % CO₂ + 85 % N₂)
  - some require also the presence of formate or hydrogen
- **Energy uptake**
  - the metabolism of aminoacids and intermediates of tricarboxylic acids
  - no ability to ferment or oxidise saccharides
- **Oxidase positive**
  - except *C. gracilis* and some strains of *C. concisus* and *C showae*
- **Optimal growth temperature**
  - for most species - 35-37 °C
  - **for thermotolerant (thermophilic) species causing campylobacteriosis** – 41,5 °C and no growth below 25 °C
- pH range 4.9 – 9.0, optimal – 7
- 1,5 % NaCl acts bacteriocidally
- min. a_w 0.98
Campylobacter spp.

Most campylobacters naturally inhabit the gastrointestinal tracts of many wild or domestic animals and birds

Species connected mainly to animals

- Non-pathogenic for animals and the human pathogenity is not known
  e.g. *C. hyointestinalis*, *C. isulaenigrae*, *C. lanienae C. canadensis*

- Pathogenic for animals and humans, but in humans causing disease very rarely
  e.g. *C. fetus* subsp. *fetus* - spontaneous abortions in sheep and cows (hum.: gastroenteritis)

- **THERMOTOLERANT SPECIES** (optimal growth temperature 41.5 °C)
  - Obligatory or opportunistic pathogens for human (except *C. helveticus*), but for animal host they need not to be enterepathogens (vet.:)

  **C. jejuni subsp. jejuni** – poultry, pig, cattle, sheep, cat, dog – (vet.: gastroenteritis, hepatitis in birds) – 90 % cases of campylobacteriosis

  **C. jejuni subsp. doylei** – only clinical isolates (vet.: none)

  **C. coli** – pig, poultry, cattle, sheep, birds – (vet. gastroenteritis) – 5-10 % cases of campylobacteriosis

  **C. lari** – animals, dog, cat, monkey, horses, seals, water (vet.: gastroenteritis in birds)

  **C. upsaliensis** – dog, cat - (vet.: gastroenteritis)

  **C. helveticus** – dog, cat – none – (vet.: gastroenteritis, hum.: none)

Species connected to human oral microflora

growing under anaerobic conditions (require atmospheric hydrogen)
gums disease in humans, rarely gastroenteritis

- e.g. *C. concisus, C. curvus, C. gracilis, C. rectus, C. mucosalis, C. showae, C. hominis*
Campylobacter spp.

CAMPYLOBACTERIOSIS
• passing campylobacters into small intestine
• enrichment
• adhesion and invasion on the intestinal mucosa
• toxins production
  • CDT (cytolethal distending toxin)
  • Shiga toxin
  • cholera-like enterotoxin
• spreading in lymphatic and blood system

Infectious dose in healthy adult
• app. $10^2$-$10^3$ cells in solid food
• app. 500 cells in liquid (milk, water)
  • more cells survive rapid passing through the stomach into the small intestine

Time of incubation: 1-3 days
Lasting: 7-10 days
Watery or bloody (up to 15 % of cases) diarrhea
Abdominal pain, fever, headache, vertigo, myalgia, shivers, vomiting
• Children below 1 year – often vomiting without fever, blood in stool (92 %)
Rehydration and relaxing in bed
Antibiotics only at severe progression

http://www.fao.org/docrep/007/y4722e/y4722e03.gif
Campylobacter spp.

Postinfectious complications: Guillain-Barré syndrom (GBS) = autoinunyte disease

30-50% of all GBS cases (1-2/100 000 people) are linked to C. jejuni infection

Guillain-Barre’ syndrome follows about 0.1% of Campylobacter infections

AIDP

- the most often
- sensory problems, pain
- quick developing weakness in the legs and hands
- respiratory difficulties, or muscles paralysis
- if recognized early and without complication it can be cured

http://www.bio.davidson.edu/courses/immunology/students/spring2006/blumer/gbs.html
In the last 10 years campylobacteriosis has become the most often gastroenteritis in the industrial developed countries.

The higher incidence is also related to most improved detection methods. The incidence is with seasonal peaks (the higher at the late spring and in summer – barbecuing).


The higher incidens is also related to most improved detection methods. The incidence is with seasonal peaks (the higher at the late spring and in summer – barbecuing).
Campylobacter spp.

- Industrially developed countries
  - Gastroenteritis usually in young adults and adults
- Developing countries
  - Border spectrum of infection symptoms from serious inflammatory disease to mild diarrhea or asymptomatic state carrier
  - Gastroenteritis common in children, specially below 2 years, when in relation with no neglecting morbidity and mortality
  - In adults gastroenteritis more rare as the consequence of the acquired immunity, but asymptomatic state carrier can be detected

Campylobacteriosis in EU, 2007 (campylobacteriosis – totally 200 507 cases, salmonelosisis totally 151 995) – also depends on the hygienic surveillance

**Campylobacter spp.**

Sources of infections

**FOOD (dominant)**
- Meat and meat products
  - **poultry**, cattle and pig meat
- Raw milk and milk products
- Water
- Seafood, mushrooms, rarely raw vegetables
- Not enough heated processed
- Cross-contamination
  - Storage in the fridge
  - Contamination of working surfaces and utensils
  - Direct transfer of contamination by hand

**DIRECT CONTACT** (rare)
- with contaminated animals (pets), human-human transmission

**ENVIRONMENTAL SOURCES**
- in some cases according to genotyping
- not known the source
- unfavourable conditions – changes in physiology – „viable but non-culturable“ state (VBNC)
  - resuscitation occurs in animal/human host

Campylobacters are not able to multiply in food, only to survive. Their occurrence in food has not yet been regulated by Reg. 2073/2005.
Ways to avoid Campylobacter Food Poisoning
1. Cover and chill raw chicken – Cover raw chicken and store at the bottom of the fridge so juices cannot drip on to other foods and contaminate them with food poisoning bacteria such as campylobacter.
2. Don’t wash raw chicken – Thorough cooking will kill any bacteria present, including campylobacter, while washing chicken can spread germs around the kitchen by splashing.
3. Wash used utensils – Thoroughly wash and clean all utensils, chopping boards and surfaces used to prepare raw chicken. Wash hands thoroughly with soap and warm water after handling raw chicken. This helps stop the spread of campylobacter by avoiding cross contamination.
4. Cook chicken thoroughly – Make sure chicken is steaming hot all the way through before serving. Cut into the thickest part of the meat and check that it is steaming hot with no pink meat and that the juices run clear.

http://www.redcat.gb.com/blog/
**Campylobacter spp.**

Positive incidence of *Campylobacter* spp. according to sources (%) in EU, 2007 (Member State-MS)


Occurrence of *Campylobacter* in fresh chicken meat in the market of EU (http://www.efsa.europa.eu/en/scdocs/doc/223r.pdf)

**Poultry:**
Very high contamination – specially *C. jejuni*
Colonisation in caecum up to $10^{10}$ CFU/g is asymptotic
Horizontal transmission in the herd by the faecally-oral way

**Influence of food processing technology**
contamination during slaughtering and food processing
the ability of campylobacters to survive differ according to used technologies and food matrices
**Vibrio parahaemolyticus**

**Family:** Vibrionaceae

- Gram-negative pleomorphic (curved or straight) short rod
- facultatively anaerobic
- catalase positive, oxidase-positive
- motile with sheathed, polar flagella
- growth temperature: 5-43 °C, optimum around 37 °C
- growth pH:
  - generally acid sensitive, but also know to grow at 4.5-5.0
  - optimum 7.5-8.5, grow till 11.0
- Heat sensitive – slightly higher resistance
  - $D_{49}\text{ in clum slurry} = 0.7\text{ min}$
- Need optimally 3 % NaCl for growth (0.5-8 %)
- Minimum aw: 0.973 – 0.986
- Grow very quickly

**Table 7.9 Vibrio species associated with human diseases**

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. cholerae, O1</em></td>
<td>Cholera, wound infection</td>
</tr>
<tr>
<td><em>V. cholerae, non-O1</em></td>
<td>Diarrhoea, gastroenteritis, wound infection, secondary septicemia</td>
</tr>
<tr>
<td><em>V. mimicus</em></td>
<td>Diarrhoea, gastroenteritis, wound infection</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>Gastroenteritis, wound infection, otitis media</td>
</tr>
<tr>
<td><em>V. fluvialis</em></td>
<td>Diarrhoea</td>
</tr>
<tr>
<td><em>V. furnissii</em></td>
<td>Diarrhoea</td>
</tr>
<tr>
<td><em>V. hollisae</em></td>
<td>Diarrhoea</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>Wound infection, primary septicemia, secondary septicemia</td>
</tr>
<tr>
<td><em>V. alginoleticus</em></td>
<td>Wound infection, otitis media</td>
</tr>
<tr>
<td><em>V. damsela</em></td>
<td>Wound infection</td>
</tr>
</tbody>
</table>

[http://www.biken.osaka-u.ac.jp/english/topics_e/3094.html](http://www.biken.osaka-u.ac.jp/english/topics_e/3094.html)

**Vibrio parahaemolyticus**

- **V. parahaemolyticus** in coastal areas are likely to be part of the natural flora of fish caught in coastal regions, including squid, mackerel, tuna, sardines, crab, shrimp, and bivalves, such as oysters and clams.
- Outbreaks along coastal regions during the summer and early fall (higher water temperatures)
- Cross-contamination in the fish market
- Occasional outbreaks in the USA and Europe, but in Japan the commonest cause of food poisoning (consuming raw or partially cooked fish)

**V. cholerae**
- Waterborne infection (cholera)
- Food contaminated by contact with contaminated water can often serve as the vehicle

**V. vulnificus**
- Infection associated with raw oysters
- Susceptible individuals and the immunosuppressed should avoid consumption of uncooked shellfish

**V. parahaemolyticus** infection
- Incubation period ~24 hours
- Symptoms typically resolve within 72 hours (up to 10 days in immunocompromised individuals)
- Watery diarrhea accompanied by nausea, vomiting, abdominal cramps, sometimes fever
- Usually self-limiting, treatment is not typically necessary
- In severe cases, fluid and electrolyte replacement is indicated
- Correlated with production of thermostable direct hemolysin (Vp-TDH) - deletion of the Vp-TDH gene results in loss of enterotoxic activity in laboratory models
  - The beta-hemolysis on a modified blood agar (Wagatsuma agar) - the Kanagawa phenomenon (region in Japan)
  - Kanagawa-positive strains (96 percent of Japanese clinical isolates, only 1 percent of environmental strains) produced diarrhea, while Kanagawa-negative strains did not
Bacillus cereus

Physiological and biochemical properties:
• Gram-positive, spore-forming rods
• facultatively anaerobic
• large vegetative cells typically 1.0 µm 3.0–5.0 µm in chains
• spores are central, ellipsoidal in shape and do not cause swelling in the sporangium
• growth temperature: 8 - 55 °C, optimum 28–35 °C
• any marked tolerance for low pH (min. 5.0–6.0, depending on the acidulant) or water activity (min. ~0.95)
• The spores show a variable heat resistance; recorded D values at 95 °C in phosphate buffer range between around 1 min up to 36 min. Resistance appears to vary with serovar.

Occurrence
• widely distributed in the environment (ubiquity)-soil-dwelling saprophytes
• isolated from soil, water and vegetation, a common component of the transient gut flora in humans
Bacillus cereus group

**Bacillus cereus group** - soil-dwelling saprophytes, but on occasion these bacteria can cause a wide range of diseases in humans, including food poisoning, systemic infections and highly lethal forms of anthrax

**Controversial taxonomic history based only on some very specific features**

*B. anthracis* - the causative agent of anthrax
*B. cereus* - a soil-dwelling saprophyte, causing two distinct forms of food poisoning with symptoms of vomiting or diarrhoea
*B. mycoides* – a non-pathogenic species with distinctive rhizoidal colony formations
*B. thuringiensis* - usually defined as strains that contain parasporal crystal proteins and may be toxic to insects or other invertebrates
*B. weihenstephanensis* – encompasses strains with a psychrotrophic physiology

Phenotypic and classical DNA hybridization studies have failed to clearly separate these taxa - **they can be considered a single species.**

But the pathogenic properties of *B. anthracis* and the biocontrol applications of *B. thuringiensis* have outweighed pure taxonomic considerations and **the separate species have been retained.**

### Bacillus cereus

**Table 7.2 Characteristics of the two types of disease caused by Bacillus cereus**

<table>
<thead>
<tr>
<th></th>
<th>Diarrhoeal syndrome</th>
<th>Emetic syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infective dose</strong></td>
<td>$10^5$–$10^7$ (total)</td>
<td>$10^5$–$10^8$ (cells g$^{-1}$)</td>
</tr>
<tr>
<td><strong>Toxin produced</strong></td>
<td>In the small intestine of the host</td>
<td>Preformed in foods</td>
</tr>
<tr>
<td><strong>Type of toxin</strong></td>
<td>Protein(s) 3 components</td>
<td>Cyclic peptide MW 1.2 kDa</td>
</tr>
<tr>
<td></td>
<td>MW37, 38, 46 kDa</td>
<td></td>
</tr>
<tr>
<td><strong>Heat stability</strong></td>
<td>Inactivated 56°C, 5 min</td>
<td>Stable 126°C, 90 min</td>
</tr>
<tr>
<td><strong>pH stability</strong></td>
<td>Unstable &lt;4 and &gt;11</td>
<td>stable 2–11</td>
</tr>
<tr>
<td><strong>Incubation period</strong></td>
<td>8–16 h (occasionally &gt;24 h)</td>
<td>0.5–5 h</td>
</tr>
<tr>
<td><strong>Duration of illness</strong></td>
<td>12–24 h (occasionally several days)</td>
<td>6–24 h</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td>Abdominal pain, watery diarrhoea and occasionally nausea</td>
<td>Nausea, vomiting and malaise sometimes followed by diarrhoea, due to additional enterotoxin production?</td>
</tr>
<tr>
<td><strong>Foods most frequently implicated</strong></td>
<td>Meat products, soups, vegetables, puddings/sauces and milk/milk products</td>
<td>Fried and cooked rice, pasta, pastry and noodles</td>
</tr>
</tbody>
</table>


Adams and Moss, 2008
Bacillus cereus

Diarrhoeal syndrome

• resemble those of Clostridium perfringens food poisoning
• production in the gut of two three-component enterotoxins:
  • a haemolytic enterotoxin HBL (consisting of three proteins B, L1 and L2)
  • a non-haemolytic enterotoxin NHE
• some strains produce both HBL and NHE, others only one
• the toxins, which are sensitive to heat and proteolytic enzymes such as trypsin and pepsin, are produced in the late exponential/early stationary phase of growth
• effect by binding to epithelial cells and disrupting the epithelial membrane
• the toxins can be produced in food, but their sensitivity to low pH and proteolysis, and the relatively long incubation period associated with illness indicate that toxin production in the gut is primarily responsible for the observed symptoms.
Bacillus cereus

Emetic syndrome
• resembles the illness caused by Staphylococcus aureus
• caused by emetic toxin preformed in foods
• emetic toxin – cereulide
  • a 1.2 kDa cyclic peptide
  • acid and heat resistant
  • produced in the food in the late exponential to stationary phase of growth
• to act by binding to and stimulating the vagus nerve

http://www.chiralix.com/rightclick.cfm?id=67352#

cereulide
cyclo[-Val-1\textsuperscript{13}C-D-O-Leu-D-Ala-3\textsuperscript{13}C-O-Val-]\textsubscript{3}
**Bacillus cereus**

- Spores resistant to drying and heating
  - food-poisoning bacilli are widely distributed in foods, but only as a small part of the total flora
- Selection of spore formers by heating
- Spores germination and outgrowth, when storage at inadequate chill temperatures, especially of the high $a_w$ (sauce at ambient temperature)

**Emetic syndrome**
- starchy products such as rice and pasta dishes
- typical: rice is prepared in bulk, in advance
- chilling to below 8 °C need not to be efficient due to the slow rate of cooling in the centre of a bulk of cooked rice
- reheating the rice prior to serving will not inactivate the emetic toxin

**Diarrhoeal syndrome**
- meat products, soups, vegetables, puddings and sauces, honey
- dried herbs and spices used in food preparation

**Milk and dairy products:**
- isolated from pasteurized milk or cream stored at inadequate chill temperatures, but rarely associated with illness – toxin production is not favoured
**Bacillus cereus**

![Diagram of Bacillus cereus life cycle](http://img.medscape.com/article/770/540/770540-fig2.jpg)

Source: J Am Board Fam Med © 2012 American Board of Family Medicine
**Clostridium perfringens**

- Family: *Clostridiaceae*
- Gram-positive rod forming oval subterminal spores rods, relatively large rods (9-13 µm)
- encapsulated, non-motile
- anaerobic (but it can survive in the presence of oxygen)
- catalase-negative
- growth temperature: 12-50 °C, optimum 43-47 °C
- vegetative cells:
  - heat sensitive $D_{60}$ (in beef) = few minutes
  - minimum pH 5, optimum 6.0-7.5
  - minimum aw 0.95-0.97
- spores
  - heat resistant $D_{60} = 0.31$ min-38 min (inter-strain variation)
Clostridium perfringens

Classification into five types (A–E)
- based on the production of 4 major exotoxins (a, b, e, i) and 8 minor ones

**TABLE I** – Diversity of *Clostridium perfringens* toxinotypes and associated diseases (Petit, Gibert, Popoff, 1999)

<table>
<thead>
<tr>
<th>Toxinotype</th>
<th>Major Toxins</th>
<th>Minor Toxins</th>
<th>Associated diseases</th>
<th>Humans</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>++</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>ε</td>
<td>i</td>
<td>CPE</td>
<td>λ</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

-- no detected toxin production; + – detected toxin production; ++ – highest toxin producer

- **C. perfringens** type A – cause of most food poisoning (and gas gangrene)
  - produces only the a major toxin with lecithinase (phospholipase C) activity
    - the ability to hydrolyse lecithin - an important role in the pathogenesis of gas gangrene
    - no role in the food poisoning syndrome
  - type A is widespread in the environment
    - in soil - at levels of $10^3$–$10^4$ CFU/g
    - isolated from water, sediments, dust, raw and processed foods
    - a common inhabitant of the human gastrointestinal tract
      - spore counts of $10^3$–$10^4$/g common in faeces from healthy individuals
  - types B,C,D, and E are obligate animal parasites of more limited distribution.
**Clostridium perfringens**

*Clostridium perfringens* enterotoxin
- a 35 kDa protein
- inactivated by heating in saline at 60 °C for 10 min
- sensitive to some proteolytic enzymes
- acting like cholera toxin by reversing the flow of Na⁺,Cl⁻, and water across the gut epithelium from absorption to secretion
- synthesized by the sporulating cells

*Food poisoning*
- a self-limiting, non-febrile illness
- nausea, abdominal pain, diarrhoea and, less commonly vomiting
- usually caused by sporulation of ingested vegetative cells in the small intestine
- incubation period: 8 - 24 h
- in healthy individuals no required medical treatment
  - occasional fatalities in the very old or debilitated
  - recovery complete within 1–2 days.
- rarely caused directly by ingestion of pre-formed toxin
- incubation period less than 2 h

The typical scenario of *C. perfringens* food poisoning
- a meat dish cooked in advance and then subjected to temperature/time abuse and served cold or reheated insufficiently to kill the vegetative cells
- ~the spores survive the cooking and in the ideal conditions without competitive microflora multiply rapidly, not destroyed later)

*Food*
- meat products such as stews, meat gravies, roast joints and pies (but usually not cured meats)
  - the frequent association of *C. perfringens* with meats, but the major contributory factors
  - the low redox potential
  - mode of preparation and consumption
- most outbreaks - in connection with institutional catering such as schools, old people’s homes and hospitals.
**Clostridium botulinum**

- **Family:** Clostridiaceae
- **Gram-positive** straight or slightly curved rods (2–10 µm long) rod forming central or subterminal oval spores
- **encapsulated, motile** with peritrichous flagella
- **obligately anaerobic**
- **division into four groups (I, II, III and IV)** due to the physiological diversity of strains
  - in reality single strains are so physiologically and biochemically different not to fulfill the single species demand, but it is accepted to avoid any possible confusion with fatal consequences if reclassified
- the most important common feature = production of pharmacologically similar neurotoxins causing botulism
- **eight serologically distinct toxins** are recognized
  - A, B, C1, C2, D, E, F, and G
    - though C2 is not a neurotoxin
- single strain usually produces only one type
Most cases of botulism in humans are due to toxin types A, B or E

**C. botulinum**
- essentially a soil saprophyte
- occurring widely
- most often isolated from e.g. aquatic muds (a moist, anaerobic, nutrient-rich environment)
- occasionally found to grow in the alimentary tract of birds and mammals
**Clostridium botulinum**

**Growth pH**
- the minimum pH depends on factors such as temperature, water activity and the acid,
- the consensus has long been a pH around 4.7 as absolute minimum
  - important for the canning industry
- non-proteolytic strains lower acid tolerance (inhibited at pH 5.0–5.2)
- but also report the growth at pH values as low as 4.0 in protective, high-protein containing media (not the situation of canning industry)
- the maximum pH for growth is 8.5–8.9 and the toxin is unstable at alkaline pH values
- (possible protective factors in fermented fish products)

**Botulism**
- three types
  - **foodborne botulism** (only in this type food is invariably involved)
    - bacterial food poisoning
    - the ingestion of an exotoxin produced by *Clostridium botulinum* growing in the food
    - the botulinum toxins are neurotoxins; they affect primarily the cholinergic nerves of the peripheral nervous system.
  - infant or infectious botulism
  - wound botulism
**Clostridium botulinum**

**the botulinum toxins**
- 150 kDa proteins
- the most toxic substances known
- a lethal dose for an adult human $\sim 10^{-8}$ g
- inactivated by heating at 80° C for 10 min
- produced during logarithmic growth as complexes
- to mitigate the effect of toxin already adsorbed at the neuromuscular junction is almost completely impossible (some neuromuscular blockade antagonists have produced transient improvements)
**Clostridium botulinum**

- rare but serious foodborne disease
- initial symptoms: from 8 h to 8 days, most commonly 12–48 h
- vomiting, constipation, urine retention, double vision, difficulty in swallowing (dysphagia), dry mouth and difficulty in speaking (dysphonia)
- fatal cases - respiratory or heart failure (1-7 days after) under the patient remains conscious
- mortality rate 20-50 %, dependent on the type of toxin (type A usually produces a higher mortality than B or E), the amount ingested, the type of food and the speed of treatment
- surviving patients – up 8 months to recover fully
- survival is critically dependent on early diagnosis and treatment
  - alkaline stomach washing to remove any remaining toxic food
  - intravenous administration of specific or polyvalent anti-toxins to neutralize circulating toxin
  - mechanical respiratory support where necessary
Features in outbreaks of botulism
(1) The food contaminated at source or during processing with spores or vegetative cells of *C. botulinum*.
(1) The food receives some treatment that restricts the competitive microflora and, in normal circumstances, should also control *C. botulinum*.
(3) Conditions in the food (temperature, pH, *E_h*, aw) are suitable for the growth of *C. botulinum*
(4) The food is consumed cold or after a mild heat treatment insufficient to inactivate toxin.

Food
Low-acid canned foods
• stringent process control in the canning industry
• problems: home-made canned or conserved foods, inadequately processed products
  • particularly vegetables, meat product (*botulus*=sausage in latin)

Other outbreaks:
• inadequate curing of meat product (*botulus*=sausage in latin)
• insufficiently smoked fish (hotsmoked) consumed without reheating
• fish products consumed raw after a unappropriate fermentation process
• hazelnut puree (inadequately heat processed)
• potato salad (cooked potatoes stored for several days at the ambient temperature and under anaerobic conditions before further processing)
• prepacked vegetable salad (temperature abuse, anaerobic conditions – vacuum package or packing in aluminium foil)
• pasteurised carrot juice (inadequate refrigeration of the product)
Staphylococcus aureus

• Gram-positive coccus (about 1 µm in diameter)
• Cell division occurs in more than one plane to form irregular clumps (~bunches of grapes)
• gold-yellow pigmented colonies
• catalase-positive, oxidase-negative
• **coagulase positive**
• facultative anaerobes
• growth temperature 7 - 48 °C, an optimum 37 °C
• unexceptional heat resistance
  • $D_{62} = 20–65$ s, $D_{72} = 4.1$ s (in milk)
• pH range: 4.0 and 9.8–10.0, pH optimum: 6–7
• tolerance of salt: 5-7 % NaCl, some strains ~20 % NaCl
• tolerance of reduced $a_w$ (>0.83)
Staphylococcus aureus

Occurrence:
• skin, skin glands and the mucous membranes of warm blooded animals, very often of higher primates
• in humans found in 20–50% of healthy individuals nasal tract
• normally a harmless parasite
  • metabolizing skin products and possibly preventing skin colonization
  • minor skin abscesses such as boils
• opportunistic pathogen – more serious, when the skin barrier is breached or host resistance is low
• sporadically isolated from other environmental sites as soil, marine, fresh water, plant surfaces, dust and air....

Pathogenic factors in S.aureus

- Suppurative diseases
  - impetigo
  - folliculitis
- Enteritis
- Toxic shock syndrome (TSS)
- Postoperative pneumonia
- Staphylococcal scalded skin syndrome (SSSS)
- Food poisoning

http://www.hal.kagoshima-u.ac.jp/dental/Saikin/english/study1.html
**Staphylococcus aureus**

Table 1. Unique features of some common SEs.

<table>
<thead>
<tr>
<th>Staphylococcal Enterotoxin</th>
<th>Feature</th>
<th>Binding to Class II MHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEA</td>
<td>Most common toxin associated with staphylococcal food poisoning</td>
<td>Alpha and beta chains [12]</td>
</tr>
<tr>
<td>SEB</td>
<td>Studied as a biological weapon</td>
<td>Alpha chain [13]</td>
</tr>
<tr>
<td>SEC</td>
<td>Commonly isolated from animals [14]</td>
<td>Outside the binding groove on the flanking helix from the α chain [15]</td>
</tr>
<tr>
<td>SED</td>
<td>Food poisoning [16]</td>
<td>Alpha and Beta chains [17]</td>
</tr>
<tr>
<td>SEE</td>
<td>Food poisoning [9]</td>
<td>Beta chain [18]</td>
</tr>
<tr>
<td>SEF</td>
<td>Associated with toxic shock syndrome [8]</td>
<td>Binds to alpha and beta chains [19]</td>
</tr>
<tr>
<td>SEG</td>
<td>Minor role in food poisoning [10]</td>
<td>SEB-like interaction with a chain [20]</td>
</tr>
<tr>
<td>SEI</td>
<td>Minor role in food poisoning [10]</td>
<td>Beta chain [22]</td>
</tr>
</tbody>
</table>

Pinchuk *et al.*, 2010

**Staphylococcal food poisoning**

- food-borne intoxication
  - 1-8 hours after eating (30 minutes is also possible)
  - severe nausea, retching, vomiting, stomach cramps, and diarrhea
- caused by staphylococcal enterotoxins
  - resistant to heat
  - stable over a wide pH range
  - resistant to gastrointestinal and other proteases

[http://www.slideshare.net/FarirahNoi/staphylococcal-food-poisoning-24597599](http://www.slideshare.net/FarirahNoi/staphylococcal-food-poisoning-24597599)

[http://femsre.oxfordjournals.org/content/36/4/815/F1.large.jpg](http://femsre.oxfordjournals.org/content/36/4/815/F1.large.jpg)
Staphylococcus aureus

Food
• Presence of small numbers on foods is not uncommon, but it is a poor competitor, so they are suppressed in growth
  • Naturally in poultry and raw meats
• Raw milk - mastitis in the producing herd
• Contamination by food handlers

Outbreks
• Present of enterotoxin producing S. aureus + conditions for their production
• dried milk and chocolate
  • growth and enterotoxin production in the raw milk, enterotoxins survived pasterization
• poultry products and cold, cooked meats
• salted meats
  • inhibits a large proportion of the competitive flora, while S. aureus is not unaffected
• buffet meals
• prepared advance, stored at ambient temperaturus or inadequatelly chilled
• canned foods
  • competitor free environment)
• hard cheese, cold sweets, custards, cream-filled bakery products (ice-cream)

http://www.slideshare.net/FarirahNoi/staphylococcal-food-poisoning-24597599
Listeria monocytogenes

• Gram-positive rod (0.4–0.5 µm x 0.5–2.0 µm)
• cultured at 20–25 °C possess peritrichous flagella a characteristic tumbling motility
• facultatively anaerobic
• growth temperature: 0–42 °C, an optimum 30 - 35 °C
• the heat resistance similar to that of other non-sporeforming Gram-positives (D_{60} ~ a few minutes, D_{70} ~ a few seconds)
• growth pH: inhibited below 5.5
  • the minimum growth pH dependent on both strain and acidulant (between 5.6 and 4.4)
• quite salt tolerant
  • able to grow in 10% NaCl
  • to survive for a year in 16% NaCl at pH 6.0
• catalase positive, oxidase-negative
Listeria monocytogenes

Genus Listeria has six species, two of which are pathogenic:
- L. monocytogenes – responsible for listeriosis
- L. ivanovii – an animal pathogen
- L. innocua, L. seeligeri, L. welshimerii, L. grayi

**Listeria monocytogenes** produces a listerolysin O
- 58 kDa b-haemolysin,
- acts synergistically with the haemolysin produced by *Staphylococcus aureus* = enhanced haemolysis on blood agar
- the CAMP test after Christie, Atkins, and Munch-Peterson
**Listeria monocytogenes**

**Occurrence**
- ubiquitous in the environment
- isolated from fresh and salt water, soil, sewage sludge, decaying vegetation, and silage

**Food**
- able to grow on most non-acid foods
- relatively resistant to curing ingredients
- outbreaks
  - raw vegetables
  - Delicatessen
  - smoked seafood
  - raw and pasteurized milk (cross-contamination)
  - **soft cheeses**
    - survives poorly in unripened soft cheeses (cottage cheese)
    - survives well in ripened products (type Camembert, Brie)
    - microbial utilization of lactate during ripening process - released amines increase the surface pH – Listeria is allowed to multiply to dangerous levels.
**Listeria monocytogenes**

Facultative intracellular pathogen
- survive and multiply in cells of the monocyte–macrophage system
- **penetrates** the gut either by crossing the Peyer’s patches or by invading enterocytes
- **endocytosis** is promoted by virulence factors (internalin, listeriolysin O etc.)
- **releasing and replication in the cytoplasm**
- only 10% of those invading monocytes are successful
- **transfer** to another cell by using **host actin filaments** polymerized around, avoiding the host’s immune system
- **dissemination** around the body from the mesenteric lymph nodes **via the blood**
- **the liver**
  - elimination and control of the infection
  - infected hepatocytes - intense inflammatory reaction - destroyed by neutrophils together with the released bacteria
- Infection not under control in the liver – dissemination by the blood to the central nervous system or placenta.
Listeria monocytogenes

If Listeria food poisoning occurs...

Listeriosis
Incubation periods:
1-90 days, typically of a few weeks
Symptoms:
varies from a mild, flu-like illness to meningitis and meningoencephalitis

most likely to develop in pregnant women
• mostly an influenza-like illness (fever, headache), occasional GI symptoms
BUT transplacental foetal infection
(abortion, stillbirth, or premature labour),

the elderly, the immunocompromised
(non-pregnant adults)
septicaemia, meningitis,
meningoencephalitis, endocarditis.
a high mortality rate 13 - 34%
early treatment with antibiotics is essential, but in the most severe forms, the prognosis remains poor

the very young
in the newborn:
an early-onset syndrome (mortality up to 38 %)
pneumonia, septicaemia and widely disseminated granulomas
a late-onset syndrome (mortality up to 26 %)
meningitis is more common
Viral foodborne infections

Norwalk virus (Norovirus)
Group IV (+)ssRNA

Hepatovirus A
Group IV (+)ssRNA

Rotavirus
Group III (dsRNA)

Only a few viral particles are necessary for the disease to develop.
High numbers of viral particles are further transmitted via feces of infected persons
(up to $10^{11}$ particles per gram of feces).
Specific lining cells are necessary for virus replication. Accordingly they cannot
multiply in foods or water.
Food borne virus are relatively stable and acid resistant outside host cells.
Noroviruses are perhaps the perfect human pathogens. These viruses possess essentially all of the attributes of an ideal infectious agent: highly contagious, rapidly and prolifically shed, constantly evolving, evoking limited immunity, and only moderately virulent, allowing most of those infected to fully recover, thereby maintaining a large susceptible pool of hosts.

-Aron J. Hall, CDC
FOOD SAFETY RULES

https://cheflynnie.files.wordpress.com/2013/03/safety.jpg

http://tx.english-ch.com/teacher/christin/foodsaferules.jpg
FOOD SAFETY RULES

- The temperature danger zone is between 5°C and 60°C, when it is easiest for harmful bacteria to grow in food.
- Minimise the time that food spends at these temperatures in order to keep food safe.
- Refrigerated food needs to be kept at 5°C or below.
- Hot food needs to be kept at 60°C or above.

Temperature danger zone:
- Hot food zone: 100°C (Bacteria are destroyed).
- 60°C (Bacteria grow quickly).
- 5°C (Cold food zone).
- 0°C (Frozen food zone: Bacteria don't grow at -10°C).

Cross Contamination: Food Storage
In order to store food healthily without contamination, a certain standard of food storage should be maintained:

www.eskyweb.com


http://www.eskyweb.com/files/image/images/esky-food-storage-preview.png
Literature:


Thank you for your attention

Questions to be sent to
Sabina.Purkrtova@vscht.cz