Types of bacterial toxins, biogenic amines and food spoilage microflora

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• food contains natural chemicals, including carbohydrates, sugars, proteins and vitamins
• not sterile, contains microorganisms
• some foods contain potentially harmful natural toxins such as mycotoxines, shigatoxines, enterotoxines etc.
Food safety


• "Foodstuffs should not contain micro-organisms or their toxins in quantities that present an unacceptable risk to human health"

• In Czech Republic EC Regulation replaced the Act No. 110/1997 Coll., on food and tobacco products and on amendments to certain Acts.
Commission Regulation on microbiological criteria for foodstuffs

- foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk to human health

- Chapter 1. **Food safety criteria**

- Chapter 2. **Process hygiene criteria**

  - *Listeria monocytogenes* less than 100 KTJ/g
  - *Enterobacteriaceae, E. coli*, Shiga toxin producing
    - *E. coli (STEC)* O157, O26, O111, O103, O145 and O104:H4
  - *Salmonella* spp.
  - Coagulase-positive *staphylococci*, St. enterotoxins – *St. aureus*
  - *Cronobacter (Enterobacter) sakazakii*
  - Presumptive *Bacillus cereus*
  - Histamine
Foodborne disease

**Bacterial infection**
Portable (communicable) ➔ fecal oral transfer

- salmonellosis ➔ *Salmonella*
- campylobacteriosis ➔ *Campylobacter*
- abdominal tyf ➔ *Salmonella typhi*
- dysentery ➔ *Shigella dysenteriae*

**Bacterial intoxication**
Non-communicable ➔ toxins - contained in food
- formation in the digestive tract

- *Clostridium perfringens* ➔ forming toxin in the small intestine
- Staphylococal enterotoxicosis ➔ thermostable enterotoxins boiling withstand 15 min
- *Clostridium botulinum* ➔ heat-labile toxin (boiling 15 min destroying it)
  ➔ neurotoxin - paralysis of respiratory muscles
Typical pathogenic bacteria

Literature and sources used
http://cwx.prenhall.com/bookbind/pubbooks/brock/chapter19/objectives/deluxe-content.html
Bacterial toxins

1. Endotoxins (bound per cell)
2. Exotoxins (secreted into the environment)
3. Virulence factor

ad 1) **endotoxins** particularly in Gram-negative bacteria (such as *Salmonella, Shigella, E. coli*), a **polysaccharide nature**, are part of the bacterial cell wall

ad 2) bacterial **exotoxins are mostly protein** in nature - inactivated by prolonged heating

Food may be formed:
- Botulinum toxin (*Clostridium botulinum*) = botulin = „sausage poison“
- *S. aureus* enterotoxins

ad 3) Listeriolysin O
Microbial intoxication

1. Bacterial toxins (endotoxins, exotoxins or virulence factors)
   - neurotoxins (*Clostridium botulinum* - botulinum toxins)
   - toxins affecting membrane (*Staphylococcus aureus, STx E. coli*)
   - toxins causing lesions (*Clostridium perfringens* - enterotoxins, *Bacillus cereus*)
   - immunoactive -endotoxin (*Salmonella abortus*, Gram-negative bacterial toxins)

2. Toxins algae

3. Mycotoxins

4. Toxic metabolites
Bacterial exotoxins

- First bacterial virulence factors identified
- Diphtheria toxin isolated in 1888

1. Bacterium produces and releases exotoxin.
2. B (binding) component of exotoxin binds to host cell receptor and exotoxin enters cell.
3. A (active) component of exotoxin alters cell function by inhibiting protein synthesis.

Literature and sources used
http://www.docstoc.com/docs/83119095/Bacterial-Exotoxins
Bacterial toxins

• were discovered in the 19th century
• are among the strongest acting toxins in nature
• **botulinum toxin and tetanus toxin (tetanotoxin)** are most strongly engaged at all, so they are referred to as "supertoxins". The toxins are **released into the food** during the bacterial growth in contaminated food. They affect mainly the nerve cells (called **neurotoxins**)
• into the body the toxins bacteria ingestion of contaminated food received. For consumers, it can cause food poisoning from the food-borne intoxication
• toxins that damage the cells of the intestine and the affected individual cause **diarrhea and vomiting**, are referred to as the **enterotoxins**. The disease is then called „enterotoxicosis“
• among the most famous producer of **enterotoxicosis** (intestinal diseases caused by bacterial toxins) include bacteria **Staphylococcus aureus, Bacillus cereus** and **Clostridium perfringens**
• toxin production by these bacteria is **associated with the transition** from "active" forms (i.e. vegetative) to form a "sleeping" (the spore). This transition causes mainly too acidic environment and temperature increase. Conventional thermal processing of foods while reducing the number of "active" bacterial cell, but can paradoxically contribute to the production of toxins which have previously not present in the foodstuff. The same effect has also acidification of food such as adding vinegar to the mayonnaise salads
Bacterial toxins

• Cytolethal distending toxins
• Hemolysins
• Pore-forming toxins (PFTs)
• Neurotoxins
• Heat-stable enterotoxins (STs) and heat-labile enterotoxins (LTs)
Cytolethal distending toxins

- **cytolethal distending toxins** (abbreviated CDTs) are a class of heterotrimeric toxins produced by certain **gram-negative bacteria** that display DNase activity
- many of these bacteria, including *Shigella dysenteriae*, *Haemophilus dysenteriae*, *Escherichia coli*, *Salmonella enterica*, *Campylobacter upsaliensis* or *Campylobacter jejuni*, infect humans. Bacteria that produce CDTs often persistently colonize their host
- these toxins trigger G2/M cell cycle arrest in specific mammalian cell lines, leading to the enlarged or distended cells for which these toxins are named
- **affected cells die by apoptosis**
- CDT-producing bacteria are often associated with mucosal linings, such as those in the stomach and intestines, and with persistent infections
- the toxins are either secreted freely or associated with the membrane of the producing bacteria

**Literature and sources used**
http://www.pdb.org/pdb/explore/explore.do?structureId=1SR4
Hemolysins (haemolysins)

- Hemolysins (UK spelling: haemolysins) are certain proteins and lipids that cause lysis of red blood cells by damaging their cell membrane.
- Although the lytic activity of some microbial hemolysins on red blood cells may be important for nutrient acquisition or for causing certain conditions such as anemia, many hemolysin-producing pathogens do not cause significant lysis of red blood cells during infection.
- Although hemolysins are able to lyse red blood cells in vitro, the ability of haemolysins to target other cells, including white blood cells, often accounts for the effects of hemolysins during infection.
- Most hemolysins are proteins, but others such as rhamnolipids are lipid biodurfactants.
Detection of hemolysis

Many bacteria produce the hemolysins that lyse blood agar component

- The genus *Streptococcus*
  - α - hemolytic activity (partial decomposition of hemoglobin, the red blood pigment change to dye green agar around the colony becomes greenish in color)
  - β - hemolytic activity (bacteria its activities fully decomposed erythrocytes in your area, blood serum agar acquires color is transparent)
  - γ - hemolytic activity (no hemolysis, most bacteria blood agar unchanged)

Blood agar - a nutrient agar is added 5-10% defibrinated, usually a ram's blood.
Hemolysins

α-hemolysin
- secreted by Staphylococcus aureus, this toxin causes cell death by binding with the outer membrane, with subsequent oligomerization of the toxin monomer and water-filled channels. These are responsible for osmotic phenomena, cell depolarization, and loss of vital molecules (v.gr. ATP), leading to its demise.

β-hemolysin
- Upon investigating sheep erythrocytes, its toxic mechanism was discovered to be the hydrolysis of a specific membrane lipid, sphingomyelin, which accounts for 50% of the cell’s membrane. This degradation was followed by a noticeable rise of phosphoryl choline due to the release of organic phosphorus from sphingomyelin and ultimately caused cell lysis.

γ-Hemolysin
- Unlike beta-hemolysin, it has a higher affinity for phosphocholines with short saturated acyl chains, especially if they have a conical form, whereas cylindrical lipids (e.g., sphingomyelin) hinder its activity. The lytic process, most commonly seen in leucocytes, is caused by pore formation induced by an oligomerized octamer that organizes in a ring structure. Once the prepore is formed, a more stable one ensues, named β-barrel. In this final part, the octamer binds with phosphatidylcholine.

Literature and sources used
http://en.academic.ru/dic.nsf/enwiki/5448186
The Christie-Atkins-Munch-Peterson = (CAMP) test. β-hemolytic *Staphylococcus aureus* and non-hemolytic *Rhodococcus equi*. Incubation at 35°C for 24-48 h. *L. monocytogenes* (listeriolysin O - erythrocytes hemolysis) and *L. seeligeri* hemolytic reactions are slightly enhanced in the zone influenced by the *S. aureus* streak, while for *L. ivanovii* hemolytic reaction is strongly enhanced in the zone influenced by *R. equi* streak. The other species remain non-hemolytic (or weak hemolysis without synergism in *L. welshimeri*).
Pore-forming toxins (PFTs)

- **Pore-forming toxins** (PFTs) are protein *exotoxins*, typically (but not exclusively) produced by bacteria, such as *Clostridium septicum* and *S. aureus*
- are frequently cytotoxic (i.e., they kill cells), as they create unregulated *pores in the membrane* of targeted cells
- Example: **α-hemolysin** *S. aureus*

Generalized mechanism of pore formation by PFTs. Soluble PFTs bind membrane receptors, which leads to oligomerization and insertion of an aqueous pore into the plasma membrane. Note that during the oligomerization step, some PFTs remain associated with their receptor, whereas others have already disassociated at this point.

**Alpha-hemolysin from S.aureus**

*Source references and additional information can be found through the following link: [http://mmbr.asm.org/content/77/2/173/F5.expansion.html](http://mmbr.asm.org/content/77/2/173/F5.expansion.html)*
Figure 3: Structures of PFTs that are important for neonatal sepsis. Panel (a) shows available structures of cholesterol-dependent cytolysins to illustrate listeriolysins’ mechanism of pore formation. (a1) displays the crystal structure of the soluble, monomeric form of perfringolysin from Clostridium perfringens (left, PDB ID 1PFO, [57]). The cryo-electron microscopy (cryo-EM) reconstruction of the prepore (EM databank: 1106) of the listeriolysin homologue pneumolysin from Streptococcus pneumoniae displayed on the right revealed that the protomer configuration in the prepore resembles that of the soluble monomer [16]. Lipid membrane is coloured yellow. Molecular modeling of the protomer fitted into the cryo-EM pore structure below (EM databank: 1107) revealed the considerable structural rearrangements that accompany membrane pore formation. The α-helices that refold into β-sheets are coloured in red. Panel (b) shows the different structures available for ClyA from E. coli, (b1) the soluble state (PDDid 1QOY, [148]) monomer and (b2) a protomer from the dodecameric pore state, which is shown as side and top view on the right (PDB ID 2WCD, [149]). Pore-lining α-helices are in red and the β-tongue in yellow. Panel (c) shows the PFTs from S. aureus. (c1) shows from top to bottom LukF (PDB ID 1LKF, [113]), LukF-PV (PDB ID 1PVL, [114]), and LukS-PV (PDB ID 1T5R, [115]). (c2) shows the octameric pore structure of γ-hemolysin (PDB ID 3B07, [116]), protomer on the left, side and top views on the right. (c3) displays the heptameric pore structure of the AFT pore (PDB ID 7AHL, [100]), individual protomer, side and tops views. The β-stem that unfolds into the membrane lining, extended β-hairpin is shown in red.

**Literature and sources used**

http://www.hindawi.com/journals/jir/2013/608456/fig3/
Neurotoxins

• affect mainly the nerve cells
• **botulotoxin** of *Clostridium botulinum* = FOOD
  • *Clostridium botulinum* produces several exotoxins
  • the best known are its neurotoxins, subdivided in types A-H, that cause the flaccid muscular paralysis (botulism)
  • cosmetic application
• *Clostridium tetani*: produces two exotoxins, **tetanolysin** and **tetanospasmin**
  • tetanospasmin is a neutotoxin that causes the clinical manifestations of tetanus.
  • tetanus toxin is generated in living bacteria, and is released when the bacteria lyse, such as during spore germination or vegetative growth
  • coded on plasmid

**Literature and sources used**
**ST and LT toxins**

- **Heat-stable enterotoxins (STs)**
  - are secretory peptides produced by some bacterial strains, such as enterotoxigenic *E. coli*, *Staphylococcus aureus* or *Salmonella*
  - these peptides keep their 3D structure and remain active at temperatures as high as 100 °C
  - different STs recognize distinct receptors on the surface of animal cells and thereby affect different intracellular signaling pathways. For example, STa enterotoxins bind and activate membrane-bound guanylate cyclase (cGMP), downstream effects on several signaling pathways
  - these events lead to the loss of electrolytes and water from intestinal cells

*Escherichia coli*

- **heat-stable toxin 1** of entero-aggregative *Escherichia coli* (EAEC - EAST1) is a small toxin. Isolates from farm animals have been shown to carry the astA gene coding for EAST1.
- the mature STa protein from *E. coli*, which is the cause of diarrhoea in infants and travellers, is a 19-residue peptide containing three disulphide bridges that are functionally important.
- members of **heat-stable enterotoxin B** family assume a helical secondary structure, with two alpha helices forming a disulfide cross-linked alpha-helical hairpin. The disulfide bonds are crucial for the toxic activity of the protein, and are required for maintenance of the tertiary structure, and subsequent interaction with the particulate form of guanylate cyclase, increasing cyclic GMP levels within the host intestinal epithelial cells

- **Heat-labile (LT) enterotoxin**
  - is a type of labile toxin found in *E. coli*, *Bacillus cereus* and *Salmonella*

*Escherichia coli*

- the heat labile enterotoxin is inactivated at high temperatures
- it acts similarly to the cholera toxin by raising cAMP levels through ADP-ribosylation of the alpha-subunit of a GS protein leading to the constitutive activation of adenylate cyclase. Elevated cAMP levels stimulate the activation of the channel thus stimulating secretion of chloride ions and water from the enterocyte into the gut lumen. This ionic imbalance causes watery diarrhea.
Bacterial toxins
Genetic analysis

- PCR analysis, RT-PCR analysis

Multiplex PCR (mPCR) for toxin of *E. coli* O157:H7.
Preliminary detection of *E. coli* O157

- **Latex agglutination DrySpot® *E. coli* O157**
  - suspension (culture solution or phys + BHI) is pipetted to the test card DrySpot® and triturated rod
  - in the presence of antigen bound to the antibody
  - occurs to form an immunocomplex, and can be observed for agglutination and formation of blue clumps
- In a negative reaction becomes blue suspension without signs of agglutination
Direct non-competitive ELISA

a) antibody is immobilized on the surface of the microtiter plate
b) addition of sample
c) specific antibody-antigen interaction
d) "Sandwich" of the antigen and the two antibodies

usually two antibodies reactive with other determinant groups on different portions of the antigen molecule

- first Ab immobilized on the carrier surface and interacts specifically with the antigen
- the second Ab labeled with an enzyme (most often peroxidase, alkaline phosphatase, beta-galactosidase and glucose oxidase)
- after addition of a chromogenic substrate, the resulting enzymatic reaction is evaluated by spectrophotometry
**HPLC analyses**

High-performance liquid chromatography (HPLC), is a technique used to separate the components in a mixture, to identify each component, and to quantify each component.


[Diagram showing HPLC setup and chromatogram with peaks labeled 1 and 2, along with mass spectroscopic analysis.]
Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of DNA, proteins, peptides and sugars.

Identification of the delta-toxin peak using purified delta-toxin and isogenic strains.

**Literature and sources used**
Gram negative bacteria

Literature and sources used
http://healyourselighthouse.com/SUPPORTING_INFORMATION/MICROBES/BACTERIA/ABOUT/BACTERIA_characteristics_gram_pos_or_gram_neg.aspx
Toxin producing food spoilage Gram negative bacteria

- E. coli
- Shigella
- Yersinia enterocolitica and Y. pestis
- Cronobacter sakazakii
- Pseudomonas aeruginosa
- Salmonella
- Aeromonas
- Vibrio
- Campylobacter
Escherichia coli

• numerous family *Enterobacteriaceae*
• Gram negative bacteria
• rod-shaped (2-3 micron length, width 0.5 mm), slender, rounded at the ends of the cell
• facultative anaerobic metabolism (increasing the presence and absence of atmospheric oxygen)
• on the surface of one or more flagella
• occurs in the intestinal tract of vertebrates
• Some strains may be pathogenic and cause diseases such as diarrhea
• various strains of *E. coli* are differentiated by serotyping
• based on the presence of somatic (O), flagellar (H) and the capsular (K) antigens

**Literature and sources used**

Non-pathogenic strains of *Escherichia coli*

- part of intestinal microflora in humans and warm-blooded animals
- the intestine is due to *E. coli* consists of vit. K and B12, which are subsequently absorbed from the intestine
- Metabolise remnants of oxygen metabolism in the gut - preparing anaerobic environment for other strict anaerobes
- the probiotic (Nissle 1917) - prevents the penetration of pathogens to the intestinal mucosa, the use for treating disorders of the digestive system
- model organism for genetics and microbiology
- excrements can get into the external environment - an indicator of fecal contamination
- representation of non-pathogenic *E. coli* strains is approximately 95%
- according to the surface structure is divided into the serotypes

β – glucuronidase positive *Escherichia coli*
one of the serotypes of *E. coli* in the literature referred to as O157: H7 produces toxins that cause bloody diarrhea

the disease, according to a bloody speech known as hemorrhagic colitis. Its main symptoms are abdominal cramps, bloody diarrhea and a low or no fever. Infections may develop hemolytic uremic syndrome in which there is kidney failure. It is a very serious condition that especially in children and the elderly can lead to death. Treatment is very difficult, because the application of antibiotic effective against bacteria is the destruction of toxin of *E. coli* ineffective

conversely, there is an increase amount of the toxin in the body due to its extensive release from damaged bacterial cells.
Pathogenic strains of *E. coli*

- they occur in approximately 0.4-5% of the total *E. coli*
- source of infection - unpasteurized milk, inadequately cooked beef, raw vegetables, contaminated water
- as pathogens are applied by different mechanisms:

<table>
<thead>
<tr>
<th>Type</th>
<th>Toxins</th>
<th>Serotype</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterotoxigenic (ETEC)</td>
<td>LT toxin, ST toxin</td>
<td>O159:H21, O88:H25, O7:H24, O148:H28</td>
<td>The so-called travel diarrhea (Mexico, Egypt)</td>
</tr>
<tr>
<td>Enteroinvasive (EIEC)</td>
<td>not produce Shiga toxin</td>
<td>Group O167</td>
<td>Invade epithelial cells of the intestine</td>
</tr>
<tr>
<td>Enterohemorrhagic/Shigatoxigenic (EHEC/STEC)</td>
<td>Shiga toxin, verotoxin</td>
<td>O157:H7, O103:H2, O111:H8, O121:H19</td>
<td>Colonization in the colon shigatoxins - affinity to the vessel wall</td>
</tr>
<tr>
<td>Enteroaggregative (EAEC, EAggEC)</td>
<td>ST toxin, hemolysin</td>
<td>O104:H4</td>
<td>Prolonged diarrheal disease</td>
</tr>
</tbody>
</table>
A. **Enterotoxinogenic (ETEC)** cause diarrhea so-called travel. With specific protein fibers (pilus) bind to specific polysaccharide receptors of epithelial cells of the small intestine. Production of enterotoxins.

B. **B. Types of enteropathogenic (EPEC)** mainly affect the newborn, are not capable of strong bonds to the surface of epithelial cells of the large intestine cells.

C. **C. enteroinvasive (EIEC):** mechanism of action similar to *Shigella*, bacillary dysentery. Bacteria enter the cells, the production of enterotoxin leads to the destruction of the capillaries.

D. **D. enterohaemorrhagic (EHEC, shiga-like toxin = verotoxin, O157).** Bacteria adhere to the epithelium of the colon, it rapidly to invade and cause acute inflammatory reaction with tissue destruction. Gradually penetrate into the deeper tissues and blood circulation - caused hemolytic-uremic syndrome. The most common source of inadequately cooked beef.

**O157:** bacteria adhere to the epithelial cells of the large intestine, in which rapidly penetrate and induce an acute inflammatory reaction with tissue destruction.

**Literature and sources used**
http://www.bms.ed.ac.uk/research/others/smaciver/Bacteria%20Inv.htm
**Escherichia coli O157:H7**

- belongs to the group of EHEC / STEC (enterohaemorrhagic / shigatoxigenic) strains of E. coli
- It occurs mainly in the intestinal tract of cattle and sheep
- the intestinal pathogen causing bloody diarrhea (hemorrhagic colitis, hemolytic-uremic syndrome), sometimes ending up death
- the presence of *E. coli O157 : H7* was monitored in meat and meat preparations, raw milk, cheese, butter, cream, milk powder and whey, ice cream, baby food
- consists of **two strong enterotoxins**, resembling to toxin produce by *Shigella* and damaging tissue culture Vero cells - shiga toxin Stx 1 and Stx 2
- STx binds and damage the epithelium of intestinal mucosa, can penetrate into circulation
- STx have affinity to the wall of blood vessels and capillaries - after the penetration inhibiting ribosomes, avoiding the formation of proteins and the cell dies - the subsequent occurrence of blood in stool
- **treatment is very difficult** - the use of antibiotics is contra-indicated, as it increases the amount of toxin in the body (due to its wider release from damaged bacterial cells and induction of gene expression in surviving bacteria shigatoxins)
Shigatoxin

- It contains two subunits - oligomer B and enzymatically active A subunit
  - Oligomer B contains 5 identical subunits, binds to the receptor in the membrane glycolipid active cell
  - An enzymatically active A subunit penetrates the cell and affects its metabolism (inhibits protein synthesis - cell unviable)

- STx 1 is very similar to shigatoxins that produces *Shigella dysenteriae*
- STx 2 is about 1000 times more toxic than Stx 1
Escherichia coli O157:H7

- with decreasing temperature (compared with the optimum growth) occurs to decrease the production of ST toxins

- *E. coli* do not survive 16-17 seconds by heating at temperatures higher than 64.5°C

- the frozen product was observed to decrease the number of viable cells of pathogenic *E. coli* even after 9 months of storage at -20 °C and at -80°C
Shigella spp.

Bacteria of the genus *Shigella* (family *Enterobacteriaceae*):

- small, motile, gram-negative, facultative anaerobic, non-spores forming bacilli
- ferment lactose are lysine decarboxylase negative

The genus *Shigella* comprises species:

- *Shigella dysenteriae*
- *Shigella flexneri*
- *Shigella boydii*
- *Shigella sonnei*
Shigella spp.

- *Shigella* cause illness called Shigellosis or **bacillary dysentery (dysentery)**
- The **infectious dose is very low**, less than 100 bacteria
- The incubation period short, usually three days. Duration of illness 1-2 weeks
- *Shigella* invade epithelial cells, there are propagated and extend simultaneously leads to the production of **endo-and exotoxins**
- The heaviest disease causes *S. dysenteriae, S. sonnei* the mildest
- Clinical signs are from mild watery diarrhea to severe dysentery with blood, pus and mucus in stools
- Symptoms also include fever, pain, urge the stool, inflammation and ulceration of the intestinal mucosa (ulcer)
- In poorly nourished children, the elderly and immunosuppressed persons may be complications (toxic megacolon, sepsis) and death of the patient
- *Shigella* is pathogenic for humans. The source of infection is most often a **person** with acute or chronic form of disease transmission occurs via the faecal-oral transfer
- Food is not specific vector for *Shigella*, epidemics have been reported after consumption of milk and milk products, fruits and vegetables. The source may also be contaminated with water
**Yersinia spp.**

Bacteria of the genus *Yersinia* (family *Enterobacteriaceae*) are:
- Gram-negative, facultative anaerobic, short rod-shaped bacteria (coccobacilli)
- oxidase negative, ferment glucose but ferment lactose, at temperatures above 35°C loses mobility

The genus *Yersinia* includes 11 species, 3 of which are pathogenic to humans:
- *Yersinia pestis*
- *Yersinia pseudotuberculosis*
- *Yersinia enterocolitica* (O:3, O:5, O:8, O:9)

- *Yersinia* cause disease characterized by abdominal pain and fever – „*Yersiniosis““

- the **infectious dose for humans is high** - $10^9$ bacteria

- the incubation period is 24-36 hours, but was also described period lasting 11 days. The disease persists 1-3 days, exceptionally up to 14 days

- *Yersiniosis* manifested as diarrhea and abdominal pain (like for other pathogens), after penetration into the lymphatic system mimitate symptoms of acute appendicitis.
Yersinia enterocolitica

Pathogenesis:
• Prolonged infection may lead to secondary complications (inflammation of lymph nodes, septicemia, reactive arthritis, etc.).
• Most susceptible to infection are young children (especially up to 1 year of age) and seniors.

The occurrence in the environment and food:
• the primary reservoir of Yersinia are pigs (Y. enterocolitica is isolated area of the tongue, throat, intestinal contents and faeces of pigs).
• Furthermore, YE isolated from faeces of domestic, farm and wild animals.
• Yersinia contaminate especially raw vegetables, milk, dairy products, pork, etc.
• in the environment of the Yersinia occur in rivers, soil and vegetation.
Yersinia pestis

• contains body antigens, cytostatic effect, effect against phagocytes
• endotoxin **pesticin** - coagulated plasma
• the reservoir of infection is wild rodents, transmission through fleas plague

The cutaneous form:
• Bubonic form - the bubonic plague (nodal) - CNS damage
• Pulmonary form - symptoms of pneumonia - death by suffocation

Literature and sources used
Cronobacter sakazakii

- foodborne pathogen of emerging importance
- threatening infections in low birth-weight infants
- first cases in 1958
- up to 2005 75 cases of Ent.sakazakii infection worldwide
- Gram-negative, motile member of the Enterobacteriaceae
- typical mesophile, growth between 6° - 47°C
- heat resistance varies between strains
- Powder infant formula foods, water, soil, tofu
- Enterobacter till 2007, from 2007 Cronobacter – based on AFLP analysis and ribotypisation
Pseudomonas spp.

- *Gram-negative bacteria*
- Family *Pseudomonadaceae*
- containing around 200 validly described species
- the members: great deal of metabolic diversity, and consequently are able to colonise a wide range of niches

Representatives:
  - *P. aeruginosa*: an opportunistic human pathogen
  - *P. syringae*: soil bacterium
  - *P. putida* and *P. fluorescens*
  - *P. aeruginosa* flourishes in hospital environments, and is a particular problem in this environment, since it is the second-most-common infection in hospitalized patients – nosocomial infections
  - pathogenesis may in part be due to the **proteins secreted** by *P. aeruginosa*
  - bacterium possesses a wide range of secretion systems, which export numerous proteins relevant to the pathogenesis of clinical strains

[Link to image of pseudomonas](https://www.vscht.cz/main/soucasti/fakulty/fpbt/ostatni/miniatlas/pseu.htm)

**Literature and sources used**
**Pseudomonas aeruginosa**

- is a gram negative bacterium belonging to group of fluorescent pseudomonads
- as a potential pathogen causes a number of diseases such as inflammation of the urinary tract, middle ear or suppuration burns
- it is highly resistant to antibiotics and is carefully monitored in medicine, hygiene and food microbiology
- most tribes excludes highly toxic toxin A
- *P. aeruginosa* uses the virulence factor exotoxin A to inactivate ADP-ribosylate eukaryotic elongation factor 2 in the host cell, much as the diphtheria toxin does. Without elongation factor 2, eukaryotic cells cannot synthesize proteins and necrotise
- in addition *P. aeruginosa* uses an exoenzyme, ExoU, which degrades the plasma membrane of eukaryotic cells, leading to lysis

**Food spoilage**
- metabolic diversity, ability to grow at low temperatures, and ubiquitous nature, many *Pseudomonas* spp. can cause food spoilage
- examples include dairy spoilage by *P. fragi* or *P. lundensis*, which causes spoilage of milk, cheese, meat and fish
**Salmonella** spp.

- G-rod of the family *Enterobacteriaceae*
- primary occurrence in the intestinal tract of animals and human
- *S. enterica* a *S. bongori*, many serotypes
- significant reservoir – Poultry
- *Salmonella* serotype *Enteritidis* - salmonellosis
- occurrence in foods - eggs, meat, milk
Salmonella spp.

• found worldwide in both cold-blooded and warm-blooded animals, and in the environment
• cause illnesses such as typhoid fever, paratyphoid fever and food poisoning
• he organism enters through the GIT and must be ingested in large numbers to cause disease in healthy adults
• infectious process can only begin after living *Salmonella* (not only their toxins) reach the gastrointestinal tract
• after ingestion of food contaminated with *Salmonella* penetrate into the small intestine where they multiply and are then released toxic substances that penetrate into lymphatic and blood circulation
• the most important toxins include endotoxin, and to a lesser extent, ST (heat stable enterotoxins) and LT (heat labile enterotoxins) exotoxins
• the local response to the endotoxins is enteritis and gastrointestinal disorder
Aeromonas spp.

- nowadays the incidence of disease caused by Aeromonas (in particular *A. hydrophila, A. caviae* but also and *A. sobria*) to rise
- family *Aeromonadaceae*
- it is due to the ability of these bacteria to grow in cooler temperatures (refrigerator)
- these are members of the gamma - proteobacteria, so they are gram-negative, oxidase positive rods fermenting glucose
- they are moving rods placed through polar flagellum
- the optimum growth temperature is 28°C, their main habitat of various types of freshwater rivers, lakes and reservoirs
- gastroenteritis caused by *A. hydrophila*, affects mostly children under five years of age
- all members of the genus *Aeromonas* produce *cytotoxic enterotoxins*
**Vibrio spp.**

- Gram-negative, short, curved (shaped "comma" - comma) non-sporulating rods, moving polar flagella
- vibria are facultative anaerobic, family *Vibrionaceae*
- besides catalase forming oxidase and reduced nitrates. It forms a considerable amount of extracellular enzymes (proteases, amylases, lipases, phospholipases, chitinase, DNase)

**Types of Vibrio**

- *Vibrio cholerae*
- *Vibrio lumunisus, fischeri* – luminiscent
- *Vibrio fetus, paraheamolyticus*
**Vibrio cholerae**

- in the 19th century, several cholera pandemics that occurred in Europe and America
- spread from the Indian subcontinent
- cholera has become a concept, it has generated concern among the people, and testified to the **poor hygienic conditions**
- latest cholera pandemic began in 1961 on the island of Celebes and reached up to southern Europe
- it was caused by a variant of *V. cholerae El Tor*. Besides the agents of cholera (*Vibrio cholerae* and *Vibrio El Tor*) are known, other species of *Vibrio*, which may cause disease in humans, either only in the **gastrointestinal tract or systemic**
Pathogenesis

- cholera has an incubation period from one to three days
- illness vary from mild, self-limiting diarrhea to a severe life-threatening disorder
- *Vibrio cholerae* overcomes the acidic pH of the stomach
- administration of $10^6$ bacterial content with food and bicarbonate sufficient to cause disease. In the small intestine mucus penetrates participation mucinase and proteolytic enzymes and binds to enterocytes, loss of fluid and ions lead to dehydration and acidosis and thereby to death
- adhering *Vibrio* choleragen form that binds to a receptor in the cell membrane. *Vibrio* do not invasion in depth
- toxin production by the different strains is different, but the epidemic strains *always produce toxin*
- *V. cholerae* is a non-invasive toxigenic bacteria
- The main factor of pathogenicity is known as *enterotoxin choleragen (cholera toxin)*, pathogenicity is strongly linked to formation of 22 kDa *thermostable extracellular hemolysin*
  - adhesion to the epithelium of the small intestine
  - propagation and production of cholera toxin
  - stimulation of adenylate cyclase
  - cAMP accumulation
  - loss of fluid and salt (up to 1 l/hr)
**Vibrio spp.**

- cholera is regarded as a **water-borne infection**, though food which was in contact with contaminated water is also vehicle
- *Vibrio cholerae* is permanently settled in the aquatic environment in endemic areas in unfavourable conditions survives in a state where it can not be proven by culture. Under favourable circumstances, is returning to its fully active form, can be demonstrated by culture. After expansion again causes the disease and there is a new epidemic spread
- the source of infection is only **human**
- sea food, shellfish, oysters *etc.*
Campylobacter spp.

- bacteria of the genus *Campylobacter* (family *Campylobacteriaceae*) Gram negative, microaerophilic, spiral small curved rods with a characteristic movement like corkscrew
- the thermotolerant campylobacters (ability to grow at 42 °C) include *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari*
- thermotolerant *Campylobacter* known as a major producer of foodborne illness only in the last 25 years
Campylobacter spp.

- Bacteria adhere to the intestinal mucosa in the proximal part of the small intestine and produce a toxin which penetrates into the lymphatic and blood circulation.
- In some affected individuals, the disease develops only in haemorrhagic enteritis and ulcerative changes in the column.
- For liquids (water, milk) is a quick passage through the stomach and thereby increases the amount of living cells which penetrate into the small intestine where it propagated.
- Infectious dose in healthy humans is approximately $10^2$ to $10^3$ bacteria.
- The incubation period is 2-5 days frequently expressed.
- Symptoms of the disease include fever, intense abdominal pain, diarrhea.
The occurrence in the environment and food

- thermotolerant bacteria of the genus *Campylobacter* occur in the intestinal tract of domestic and wild warm-blooded animals often without clinical symptoms.
- these bacteria may be a human infected with either:
  - directly (e.g., direct contact with animals)
  - indirectly by contaminated water, food.
- *C. jejuni* is very often isolated from *poultry* and wild birds. *C. coli* in *pigs* prevails over *C. jejuni*.
- *C. lari* is isolated from *wild birds*, mostly seagulls.
- *C. upsaliensis* is isolated and rare example of faeces of pets (e.g., dogs and cats).
- **Low level of hygiene** when handling raw poultry in households and in catering operations.
- storage of poultry in the refrigerator along with other food intended for direct consumption.
- contamination of work surfaces and utensils when cutting and processing of poultry before cooking.
- due to the **low infectious dose** is possible and direct transmission by contaminated hands, e.g. in the preparation of raw meat from mother to child or playing with pets.
The occurrence in the environment and food

- optimum composition of the atmosphere for the growth of *Campylobacter* in 5% O2 + 10% CO2 + 85% N
- *Campylobacter* bacteria are sensitive to the majority of disinfecting agents, including chlorine preparations chlorination of drinking water is a good defense against disease
- drying is an effective means to eliminate *Campylobacter*
- use of organic acids reduces the number of *Campylobacter* and therefore in some states in the slaughterhouse before chilling poultry situated the surface treatment of carcasses with lactic acid or acetic acid
Gram positive bacteria

Literature and sources used
http://faculty.ccccmd.edu/courses/bio141/labmanua/lab14/diseases/enterococcus/u1fig9b.html
Gram positive bacteria

- *L. monocytogenes*
- *Staphylococcus aureus*
- *Clostridium botulinum*
- *Clostridium perfringens*
- *Bacillus cereus*
Listeria monocytogenes

- gram-positive facultative anaerobic bacterium
- resistant to environmental conditions
  - 0.5 – 45 °C, pH from 4.3 to 9.6, high concentration of NaCl (10 – 15 %)
- ubiquitous, found in the environments from there it can infect food
- it was described more than 100 years ago, but until the mid-80s was confirmed as the cause of foodborne disease in humans
- the genus Listeria covers 10 species, L. monocytogenes is the only species pathogenic for humans
- over the last 25 years, in Europe and in the USA have been some serious disease epidemic listeriosis with alimentary way of transmission. The severity of the disease is particularly high lethality rate of people with disabilities
• intracellular parasite - causative agent of alimentary disease listeriosis

• High mortality (~ 30 %) in the threaten groups
  • elder people, babies, persons with weakened or insufficiently developed immune system
  • abortions at pregnant women

http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8082
Pathogenesis

• not all representatives of the species *L. monocytogenes* are pathogenic
• the production of hemolysin (*listeriolysin O*) and phospholipase are of the important factors of pathogenicity
• in organisms sick people and animals with *Listeria* can multiply intracellularly as well
• the way of transmission is mainly alimentary, rarely contact
• the disease affects primarily persons with lowered resistance or physiological stress (pregnancy). Gastrointestinal *Listeria* penetrate into lymphatic and blood circulation
• *Listeria* exhibit high affinity to brain tissue, and pregnant uterus, the bacteria penetrate through the placenta and infect the amniotic infection of the fetus occurs
Listeriolysin O

- Listeriolysin O (LLO) is a hemolysin
- the toxin may be considered a virulence factor, since it is crucial for the virulence of *L. monocytogenes*
- is a non-enzymatic, cytolytic, thiol-activated, cholesterol-dependent, pore-forming toxic protein
- Listeriolysin O is encoded by the gene *hly*, which is part of a **pathogenicity islands** called LIPI-1
- transcription of *hly*, as well as other virulence factors of *L. monocytogenes* within LIPI-1, is activated by the protein encoded by *prfA* gene. *prfA* is thermoregulated by the **PrfA thermoregulator UTR element**, such that translation of *prfA* maximally occurs at 37°C and is nearly silent at 30°C
- 37°C is within the range of normal body temperature, PrfA protein, as well as listeriolysin O and other virulence factors regulated by PrfA, is only produced when *L. monocytogenes* is in a host
Listerioliysin O


Literature and sources used
http://www.nature.com/nrmicro/journal/v7/n9/fig_tab/nrmicro2171_F1.html
### Pathogenesis

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[Diagram showing the regulation of PrfA and inlA genes]
Pathogenesis

• the infectious dose of *L. monocytogenes* is not yet clearly specified, it is assumed that in healthy individuals with infectious dose of around $10^8$ cells at risk groups is significantly lower $10^2$-$10^3$ cells

• for listeriosis the incubation period ranges from several days to several weeks depending on the infectious dose, the virulence of the bacteria and the patient's condition

• the spectrum of clinical symptoms of listeriosis in general, the disease often take the form of mild flu-like symptoms (below the picture pharyngitis, sinusitis or tonsillitis), complicated cases transferred to meningitis and sepsis, often ends with the death of the affected person. This pathogenic agent causes disease in disadvantaged populations
**Listeria monocytogenes**

**Occurrence in food:**

- raw meat and raw milk
- certain types of cheeses (e.g. Olomouc curd cheese, cheese with mold on the surface ripened cheeses or smear)
- cooked meat products
- raw (frozen) vegetables – pizza
- *Listeria* are psychrotrophic pathogens in the range of temperatures at which retain full vitals from 0 to 50 °C and survive freezing (at cold temperatures are able to multiply) – carefully in fridge
- survive in foods having a salt concentration up to 10%
- Lactic acid bacteria **bacteriocins** and partially inhibit the growth of *Listeria* are sensitive to normal disinfectant agents
- Bacteria of the genus *Listeria* are **relatively resistant to the external environment**. Do not survive sterilization temperatures, but like other Gram positive bacteria and listeria are more resistant to higher temperatures than members of the *Enterobacteriaceae*. At high pre-pasteurization contamination of the cells can survive pasteurization process. As can be considered a safe pasteurization running at higher temperatures
Staphylococcus aureus

- commonly occurring G+ coccus (skin, hair, mucous membranes) = natural human host (20-50% of population)
- there are also dust and consequently on the tools and work surfaces and technological devices
- inflammation of the skin and soft tissues
- occurrence in foods - meat products, delicatessen, dairy products
- SA is one of the most common causes of mastitis in cows and can primarily contaminate raw milk
- risk - the production of heat stable enterotoxin (food poisoning)
Staphylococcus aureus

- Staphylococcal enterotoxins (SE) are a major cause of food poisoning outbreaks worldwide.
- Classical toxins produced by the bacteria *Staphylococcus aureus* (found in five types A to E) are people the most sensitive one.
- Enterotoxins acts locally in the intestinal mucosa, causing acceleration of bowel movements (peristalsis), and diarrhea. The first symptoms of staphylococcal enterotoxicosis (nausea, vomiting, diarrhea) takes effect after 2-6 hours after eating contaminated food.
- The source of infection is a carrier immune bearing in the throat, nose and tonsils, which it releases into its environment.
- The most commonly contaminated foods are mainly sausages, minced meat and cream sauce in which at elevated temperatures the organism easily propagated.
- Staphylococcal toxins significantly activates immune cells, resulting in destructive response of the entire immune system and the development of septic shock.
**Staphylococcus aureus**

- depending on the strain, *S. aureus* is capable of secreting several exotoxins, which can be categorized into three groups
  - **Superantigens of *Staphylococcus aureus*** have superantigen activities that induce toxic shock syndrome (TSS), this group includes the toxin TSST-1, enterotoxin B, which causes TSS associated with tampon use. Other strains of *S. aureus* can produce an enterotoxins that is the causative agent of *S. aureus* gastroenteritis. This gastroenteritis is self-limiting, characterized by vomiting and diarrhea one to six hours after ingestion of the toxin with recovery in eight to 24 hours. Symptoms include nausea, vomiting, diarrhea, and major abdominal pain
  - **Exfoliative toxins (EF toxins)** are implicated in the disease staphylococcal scalded-skin syndrome (SSSS), which occurs most commonly in infants and young children. It also may occur as epidemics in hospital nurseries. The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS
  - **Other Staphylococcal toxins** that act on cell membranes include alpha toxin, beta toxin or delta toxin and several bicomponent toxins. The bicomponent toxin **Panton-Valentine leukocidin (P VL)** is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community-associated methicillin resistant SA (MRSA) strains.
Staphylococcus aureus

- cases are often caused by strains carrying one or more of the five classical SE (SEA-SEE), although there are a few outbreaks reported to be caused by newly described SE
- to date, more than twenty Staphylococcal enterotoxins have been identified and divided into two groups according to their demonstrated emetic activity: classical SE and new SE
  - the members of group 1, classical emetic toxins designated SEA, SEB, SEC, SED and SEE, are the cause of about 95% of SFP (Staphylococcal Food Poisoning) in humans and can act as superantigens (SAg)
  - group 2 includes toxins postulated to be involved in the remaining 5% of SFP outbreaks, i.e. other recently identified enterotoxin and enterotoxin-like superantigens (SEI), designated SEF (TSST), SEG, SEH, SEI, SEJ, SEK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SER, SES, SET, SEIU, SEIUV (SEW) and SEIV
- all staphylococcal superantigens are encoded on mobile genetic elements, that include plasmids, prophages, S. aureus pathogenicity islands (SaPI), genomic islands vSa and the staphylococcal cassette chromosome (SCC) elements
- these mobile genetic elements have played an important role in the evolution of S. aureus as a pathogen
**Staphylococcus aureus**

- the bacteria were very resistant to the external environment
- well survive in:
  - dry and acidic environment
  - tolerate high levels of common salt
  - refrigerator and freezer temperatures
  - aerobic and anaerobic environments
- do not survive sterilization or pasteurization temperature
- toxins are extremely resistant!
**Clostridium spp.**

- genus *Clostridium* includes **sporulated** anaerobic bacteria
- vegetative bacteria having the shape of robust rod (older cultures are Gram-labile) are movable
- they form endospores (round and oval bulge bar)
- in nature, this species is widely dispersed, participate in the decay process. **Spores are highly resistant** to the environment
- they are a normal part of the intestinal microflora of humans and animals, but some species are pathogenic
Botulism is the most serious type of bacterial food poisoning
• in the late 18th century in Germany erupted illness in 13 people who ate the same type of blood sausage. Six people died. Although sausages were cooked and then smoked, were stored at room temperature. Local doctor revealed the source of infection, and since the sausage is Latin botulus, the disease was identified as botulism
• the isolation and identification of Clostridium botulinum occurred in 1896
• C. botulinum is a gram positive spore-forming anaerobic bacteria forming endospores positioned either centrally or subterminally
• vegetative forms have robust shape sticks 2 -10 micron long, older cultures are Gram unstable
Endospores

- It is dormant, tough, and non-reproductive structure produced by certain bacteria like *Clostridium* and *Bacillus*

The presence of spores, their shape, size and location

Bacterial spores location:
- a - central
- b - subterminal
- c - terminal
- d - clostridium
- e - plectridium

*Bacillus subtilis* and *Clostridium sporogenes*

Literature and sources used
**Clostridium botulinum**

- the most characteristic of this bacterium is the formation of **neurotoxins** (eight serologically distinct types - A, B, C1, C2, D, E, F and G) which have the ability to cause botulism, most disease in humans acting type A, B or E
- pure botulinum toxin is **thermolabile**, is inactivated by heating at 80 °C for 10 minutes. When the food before eating again without warmed up, then botulinum toxin gets into the body, causes severe **neuroparalysis called botulism**
- the lethal dose of botulinum toxin (protein neurotoxin) is set at 0.1 μg.kg - 1 body weight. Toxins cause blockage in nerve impulse transmission at neuromuscular griddles. This leads to paralysis of the affected muscle death occurs within 24 hours due to paralysis of the respiratory musculature. If the patient survives poisoning, then without any consequences
- *Clostridium botulinum* is a heterogeneous group of bacteria, a common feature is the formation of toxins distinguish different antigenic groups
- **endospores are highly resistant** to temperature (by heating survive two hours in boiling water)
- commonly occur in the GIT of humans and animals, soil and mud, water, where they can contaminate e.g. vegetables
We distinguish four types of the disease:

- foodborne botulism ("food-borne")
- botulism resulted from a injury ("wound")
- infant botulism ("infant")
- nonspecific botulism (ATB after treatment or surgery)

Symptoms of botulism occur from 8h to 8 days, most commonly 12-48 h, after consumption of the toxin/containing food, depending on the dose of the toxin.

- vomiting, constipation, double vision, difficulty in swallowing, dry mouth, difficulty in speaking
- neuromuscular blockade antagonist, 4-aminopyridine
- survival critically dependent on early diagnosis + treatment, mortality 20-50%, type of toxin dependent
- isolation and identification - long protocol using mice
- treatment consists in the administration of the earliest polyvalent antisera and respiratory support in affected patients. Mortality is currently in the early initiation of treatment of below 10%.
Clostridium botulinum

- mostly the single strain of *C. botulinum* produce one type of toxin, but have also been described strains producing more toxins
- generally psychotropic from 3 – 15°C
- foodborne botulism is an example of bacterial food poisoning in its strictest sense: it results from the ingestion of an exotoxin produced by *Clostridium botulinum* growing in the food
The occurrence in the environment and food

- the spores from the environment can contaminate food or raw materials
- the spores are not destroyed by conventional processing technology
- under favorable conditions, can cause food to germinate spores and toxin production
- **pure botulinum toxin is thermolabile**, is inactivated by heating 80 ° C for 10 minutes. Unless a food prior to consumption is heated again, the toxin is getting into the human organism
- most strains not-produce botulotoxin at temperatures below 4 ° C and pH> 4.0

**Risky foods:**
- home-canned foods and vegetables
- poorly conserved meat
- fermented food, made especially from contaminated vegetables

**Prevention:**
- heating the food products that may be contaminated with C. botulinum temperature at 90 or 121 ° C
- food preservation by reducing the pH (below 4.5), reduction in aw salting, sugaring, drying or freezing
- food storage at cooler temperatures
**Clostridium perfringens**

- *Clostridium perfringens* was described by the late 19th century as the cause of serious infectious diseases, gas gangrene
- much later was identified as the causative agent gastroenteritckých disease. This species is currently categorized into 5 types (A - E), by type exotoxin produced
- *Clostridium perfringens* is obligatory anaerobic rod forming endospores
- it is often found in the gastrointestinal tract of humans and animals (10⁶-10⁸/g) in the soil, contaminates particularly meat and vegetables
- it grows over a wide temperature range of 15-50 °C, the optimal temperature (43-47 °C) doubling time less than 12 minutes
- to produce enterotoxins occurs during sporulation MO (min. 10⁶ vegetative cells)
- depending toxins produced by *C. perfringens* strains are divided into 5 groups AE, with foodborne diseases induces type A, **thermolabile**
- **enterotoxins (alpha toxin - lecithinase)** damage the epithelial cells at the top of the villi of the intestines and prevents the absorption of glucose
- self-limiting, non/febrile illness nausea, abdominal pain, diarrhoea less commonly vomiting
- onset usually 8 to 24 h after consumption of food with large number of vegetative organism
- toxin is 35 kDa protein – inactivated by 10min heating at 60°C
**Clostridium perfringens**

- typical scenario of food poisoning: a meat dish containing spores of *C. perfringens* is cooked, the spores survive the cooking to find themselves in a genial environment without any competing microflora.

- after cooking, the product is subjected to temperature/time abuse, such as slow cooling or prolonged storage at room temperature. This allow the spores to germinate and multiply rapidly to produce a large vegetative population.

- the product is either served cold or reheated insufficiently to kill vegetative cells.

- most outbreaks: institutional catering (schools, hospitals).

- **Risky foods:**
  - cooked meat, poultry, fish, stews and roasts.

- **Prevention:**
  - thorough and complete heat treatment of food.
  - rapid and efficient cooling of food.
Genus *Bacillus*

- it contains **about 50 species**, is widespread in nature
- **rich enzyme facilities** so it is able to decompose various organic compounds. Most species have very active amylolytic enzymes, but also pectolytic, proteolytic. Some species form slimy housing polysaccharide nature that cause unwanted threadiness bread
- it has a strong Gram-positive rods with **spores** is aerobic. Very often these bacteria encountered as contaminants of substrates
- as human pathogens are given only *B. antracis* (i.e. agent of anthrax, anthrax). As the only species of the genus *Bacillus* has hemolysis), *B. cereus* (**cause of food poisoning**, nausea, watery diarrhea)
- often in dry foods such as cereals, flour. A large number of rice cooked in advance is ideal to propagate *B. cereus* "Chinese restaurant syndrome"
- *B. subtilis*, is the most prevalent species in nature, causes food poisoning and sometimes complications after eye injury
**Bacillus cereus**

- *Bacillus cereus* is mainly known as a major producer of **food spoilage**

- at the beginning of the 20th century has demonstrated the ability to produce **toxins** causing two etiologically different foodborne disease (intoxication)
  - **emetic form**
  - **diarrhogenic form**

- very typical is the appearance of the colonies on the blood agar

- in an environment of *B. cereus* is **widely dispersed**, naturally occurs in the dust, soil and materials of animal or vegetable origin
Pathogenesis - emetic form

- The disease occurs after consumption of a low molecular weight toxin
- A thermostable protein (121°C for 90 minutes) produced during the formation of spores. Its effect is similar to staphylococcal enterotoxins
- Emetic toxin producing strains belong to the most common serotypes H1, H3 and H8
- Emetic syndrome may cause a lesser extent also other species of the genus Bacillus, such as B. subtilis
- The infectious dose similar to the SA enterotoxins
- Incubation ranges from 1-6 hours after eating contaminated food
- Clinical symptoms include nausea, vomiting, and feeling restless
- Complications are rare, healing usually occurs within 24 hours
Pathogenesis - diarrhogenic form

- the disease causes enterotoxin (50,000 Da), which is formed during germination dispute. It is a thermo-labile protein is inactivated temperature of 45 °C. Its effect is similar to cholera toxin
- the incubation period is 8-16 hours
- clinical symptoms include abdominal pain, cramps and profuse watery diarrhea, vomiting and fever is rare
- the recovery occurs within 24 hours. Risk groups (weakening and aged) Severe diarrhea can lead to dehydration
- a similar syndrome may cause some strains of *B. licheniformis*

The diarrhetic syndromes observed in patients are thought to stem from the three toxins:

- hemolysin BL (Hbl)
- nonhemolytic enterotoxin (Nhe)
- cytotoxin K (CytK)

- the *nhe/hbl/cytK* genes are located on the chromosome of the bacteria
- transcription of these genes is controlled by *PlcR*. These genes occur as well in the taxonomically related *B. thuringensis* and *B. anthracis*. These enterotoxins are all produced in the small intestine of the host, thus thwarting digestion by host endogenous enzymes
- the Hbl and Nhe toxins are pore-forming toxins closely related to ClyA of *E. coli*. The proteins exhibit a conformation known as "beta-barrel" that can insert into cellular membranes due to a hydrophobic exterior, thus creating pores with hydrophilic interiors. The effect is loss of cellular membrane potential and eventually cell death
- CytK is a pore-forming protein more related to other hemolysins
Bacillus cereus

Emetic toxin producing strains:
• rice and other cereals
• pasta
• pasteurized milk puddings and cream

Diarrhogenic toxin producing strains:
• meat and vegetable dishes
• soups, sauces, stews, desserts
• the problem of long-term storage of cooked food at room temperatures
• meals must keep after cooking at 60°C and cool rapidly or freeze

• Large amounts of cooked rice for a few days in advance provides an ideal environment for the multiplication of B. cereus
• spores are able to survive precooking
• during the storage is pre-cooked rice germination and growth of spores and subsequent toxin production
• lowering the temperature below 8°C can partially prevent this process
• a major source of B. cereus can be dried herbs and spices that are used in food preparation
Bacillus subtilis

Literature and sources used
Electronic Microcospy of B. subtilis R0179. EM image courtesy of Dr. Sandy Smith, Dept of Food Science, University of Guelph, Ontario, Canada, http://commons.wikimedia.org/wiki/File:Bacillus_subtilis_R0179.jpg
Viruses

- A **virus** is a small infection agent that can replicate only inside the living cells of an organism. Viruses can infect all types of organisms, from animals and plants to bacteria and archaea.
- Viruses are considered by some to be a **life form**, because they carry genetic material, reproduce, and evolve through natural selection.
- They lack key characteristics (such as cell structure) that are generally considered necessary to count as life.
- Because they possess some but not all such qualities, viruses have been described as "organisms at the edge of life".
- Diameter of viruses: **20 – 300 nm**
- A virus has either DNA or RNA genes and is called a **DNA virus** or an **RNA virus**.
Food-borne Viruses (Enteric viruses)

- Viruses are very significantly different from all types of microorganisms.
- They do not have a cellular structure, having only one type of nucleic acid (either RNA or DNA), and are covered by protein shell or capsid.
- Their size is extremely small, only 25-300 nm, so they are not visible in an optical microscope.
- It is an obligate parasite cell, as outside the host cell does not reproduce.
- This means that the multiplication of viruses in food does not take place, and the food serving as passive carrier.
- Viruses transmitted by food are not new, but still pose a threat to food safety.
- Among the best-known viruses that cause disease include virus Hepatitis A, rotavirus, astrovirus, enteric adenovirus virus and hepatitis E.
- Viruses unlike bacteria do not cause "spoiling" foods because alter the sensory properties and do not produce other toxins, but are themselves highly infectious.
- The diagnosis is 10 to 100 viruses. Viral infection of food are caused by members of several families of viruses are mostly norovirus (NOV) and hepatitis A virus (HAV).
Norovirus

- Norovirus (previously called "Norwalk-like viruses") is an ssRNA virus of the family *Caliciviridae*
- This virus has about 90% of all epidemic nonbacterial diseases GIT worldwide
- It is responsible for 50% of all gastroenteritis from food in the USA
- Norovirus affects **all age groups**
- Is transmitted fecal contaminated food and/or water and direct personal contact
- GIT disease participate several groups of viruses, including rotaviruses, astroviruses, enteroviruses, coronavirus, toroviruses, reoviruses and adenoviruses.
- All groups of viruses are transmitted by the faecal-oral way, usually after eating contaminated food or water and may cause, depending on the condition and individual immunity various diseases such as gastroenteritis - a disease of the GIT
- The presence of microorganisms in foods are legislatively prescribed limits (Regulation 2073/2005 or 1441/2007)
Microbial intoxication

1. Bacterial toxins (endotoxins, exotoxins or virulence factors)
   - neurotoxins (*Clostridium botulinum* - botulinum toxins)
   - toxins affecting membrane (*Staphylococcus aureus*)
   - toxins causing lesions (*Clostridium perfringens* - enterotoxins, *Bacillus cereus*)
   - immunoactive-endotoxin (*Salmonella abortus*, Gram-negative bacterial toxins)

2. Toxins algae

3. Mycotoxins: many, e.g. *Aspergillus flavus*, *Fusarium* sp., *P. citrinum* or *Trichotheceium* sp.

4. Toxic metabolites
Mycotoxins

- Fungi are organisms classified in a separate kingdom Fungi
- fungi are due to their enzymatic equipment very adaptable to almost any substrate contamination - even food
- spores are unicellular or multicellular spores of molds used for their reproduction and survival
- mycoses are fungal diseases
- toxigenic fungi have the ability to produce mycotoxins (fungal toxins)
- some strains of toxigenic molds can produce simultaneously two mycotoxins, such as aflatoxins and cyclopiazonic acid produce by *Aspergillus flavus*
- not all strains of molds are toxigenic. If you were at any particular type of fungus strain previously found to produce certain mycotoxins, it is possible to treat all strains of this species potentially toxigenic, that is also capable of producing a mycotoxin
- mycotoxins are products of metabolism (metabolism) of toxigenic fungi
- they are significant natural toxins in food
*Chalara sp.*

*Penicillium expansum*
Mycotoxins

- currently, **several hundred known mycotoxins**, approximately 50 **mycotoxins** is given in causal connection with mycotoxicoses in humans and animals
- there are also significant **late toxic effects** such as **carcinogenic** (tumor origin) and **immunosuppressive** (impaired immunity and susceptibility to many diseases, especially the elderly and children)
- mycotoxins in toxicology studies were found to be in experiments on laboratory animals are carcinogenic or associated in epidemiological studies of the incidence of cancer in humans
- according to the International Agency for Research on Cancer (IARC / WHO) is currently classified as a proven human carcinogen ** aflatoxin B1**
- mycotoxicoses are acute or chronic diseases (poisoning) caused by mycotoxins
Mycotoxins - history

- the end of the 19th century - Japan - experience that yellow rice, it is necessary to expose a few days
- direct sunlight (**photolysis mycotoxin citreoviridinu**)
- 30th and 40th years of the 20th century - the mass poisoning Russia makes grain contested by fungi of the genus *Fusarium*
- 1960 - England - death of about 100,000 turkey poults after feed consumption (contained peanuts nuts from Brazil) affected by molds of the genus *Aspergillus* (aflatoxin)
Mycotoxins

- secondary metabolites of fungi have different properties, some of them found commercial application, others have beneficial health effects
- mycotoxins are **secondary metabolites of molds having toxic effects to humans or animals**
- the name is derived from "mykes" (= fungus) and "toxicum" (= poison)
- name was first used Forgacz and Carll 1955
- **antibiotics (toxic to microorganisms) x mycotoxins**
- there were identified and analyzed several hundred
- they are carcinogens, mutagens, teratogens, estrogens, etc.
- they are chemically very different. They have different functions, for example serve to deter rival body
- molecular weight is 200-500 g/mol
- **their production is very specific and often is linked only to one genus or strain**
- chemical resistance - mostly resistant to high temperatures and durable kitchen modifications. Some, however, are unstable.
Mycotoxins

- Mycotoxins are low molecular weight secondary metabolites fungi toxic to plants and warm-blooded animals (including human).
- Not all secondary metabolites are mycotoxins!
- One mycotoxin can be produced different genera (citrinin - *Aspergillus, Penicillium*, etc.).
- They are produced by various fungi growing in contaminated foods and other substrates, such as silage or forage.
- Some mycotoxins are present in the mycelium, part freely in substrate. Often very persistent.
- Currently, over 400 known mycotoxins produced a 150-species of fungi.
- Generally, higher concentrations are found in substrates higher water activity at elevated temperature.
- Occur especially at the end of the exponential growth phase.
- Diseases caused by mycotoxins are called „mycotoxicosis“.
Mycotoxins

1. **Aflatoxins** are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*
   - four different types of mycotoxins produced, which are B$_1$, B$_2$, G$_1$, and G$_2$.
   - aflatoxin B$_1$, the most toxic, is a potent carcinogen.

2. **Ochratoxin** is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form of Ochratoxin A.

3. **Citrinin** is a toxin that was first isolated from *Penicillium citrinum*, but has been identified in over a dozen species of *Penicillium* and several species of *Aspergillus*. Some of these species are used to produce human foodstuffs such as cheese (*Penicillium camemberti*), sake etc.

4. **Ergot Alkaloids** are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*, which are common pathogens of various grass species.

5. **Patulin** is a toxin produced by the *P. expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular rotting apples and figs. It is destroyed by the fermentation process and so is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals. In 2004, the EC set limits to the concentrations of patulin in food products. They currently stand at 50 μg/kg in all fruit juice concentrations, at 25 μg/kg in solid apple products used for direct consumption, and at 10 μg/kg for children’s apple products, including apple juice.

6. **Fusarium toxins** are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize. They include a range of mycotoxins.
Potentially aflatoxinogenic mold

- members of group *Flavi* – *A. flavus*, *A. parasiticus* a *A. nomius*.
- they are the producers of aflatoxins, particularly aflatoxin B1
- currently, other producers were described aflatoxins (*A. tamarii*, *A.pseudotamarii*, *A.bombycis aj.*)
Role of mycotoxins

• function of mycotoxins is not yet fully satisfactorily explained
• probably plays a role in the elimination other competing microorganisms in same environment
• metabolism regulation (inhibition of primary metabolism)
• defenses of organisms (*A. flavus* - aflatoxins and tremorgeny)
• they help parasitic fungus invasion of host tissues
Mycotoxins

• **Receive mycotoxins:**
  • most often in locations with higher temperature and humidity (favorable conditions for growth mold)

• **Exposure:**
  ◦ ingestion
  ◦ dermal
  ◦ inhalation (mycotoxins are usually non-volatile. However, they can be inhaled mold spores)
Fruits and vegetables
phytopathogenic fungi

**Botrytis allii**

**Botrytis cinerea**

**Micromycets**

Fruits and vegetables
Waste infections

**Zygomyces (Mucor sp.)**

**Thielaviopsis thielavioides**

**Saprophytic fungi**

Cladosporium sp.

Eurotium sp, Penicillium sp.
Mycotoxicoses

- **acute toxicity**
  - degeneration of the liver, kidney, CNS
  - hepatoxicity - cirrhosis, necrosis

- **chronic toxicity**
  - reduced production of milk and eggs, a small weight gain
  - suppression of immune function, increased susceptibility to infections
  - cancer and other chronic diseases
The occurrence of mycotoxins

- it is estimated that **over 25% of world reserves of grain** annually contaminated with mycotoxins
- in some areas, the contamination in roughage is over 90% of concentrates feed for calves and pigs up to 90%, poultry to 100%
- producers may be
  - Field (*Fusarium*, *Alternaria*, *Cladosporium*, *Diplodia*, *Giberella* etc.)
  - Warehouse (*Aspergillus*, *Penicillium* and others)
  - Warehouse and field (*Penicillium*, *Fusarium*)
- very often appear mycotoxins in foodstuffs during storage or in the remnants of the food after preparation
- **many of the contaminants in the home is capable of forming toxins!**
• commodities that are frequently contaminated with aflatoxins: corn, peanuts, pistachios and other nuts and cotton seeds
• depend of their age and storage conditions
The main mycotoxins in the interest of world legislation (order of importance)

1. Aflatoxins B a G
2. Aflatoxin M1 = hydroxyaflatoxin B₁
3. Patulin
4. Ochratoxin A
5. Deoxynivalenol (DON)
6. Zearalenon
7. Fumonisins
8. T-2 toxin

Aspergillus flavus

RNDr. F. Malíř, Ph.D.
Aflatoxins

- **Aspergillus flavus** – black tea, fruit tea, cumin, meal, flour, sweet paprika, black pepper, oatmeal
- Not all strains are toxigenic - usually only 60-80%. Aflatoxins in pepper, cumin and paprika
  
  - **A. tamarii** – pepper, black tea
  - **A. niger** (potential producer of **ochratoxin A**) - peanuts, black tea, fruit tea, cumin, flour, paprika, black pepper, raisins. The raisins also demonstrated ochratoxin A.
  - **A. parasiticus** a **A. nomius** not been isolated.

- Conclusion: Not every potentially toxigenic strain is actually a producer of mycotoxins. Compliance with the right procedure and the food itself are often barriers to the production of toxins.

![Bar chart showing frequency of aflatoxins in different types of food](image_url)
Aspergillus acidus from Puerh tea and black tea does not produce ochratoxin A and fumonisin B2.

Puerh tea is a unique Chinese fermented tea. Unlike other teas it is stored for a long period of time. Aspergillus niger is claimed to be the dominant microorganism in the Puerh tea manufacturing process and also to be common on tea in general. A. niger sensu stricto is known to produce the mycotoxins ochratoxin A, fumonisin B(2) and B(4). With this in mind, we performed a preliminary study to determine if production of these mycotoxins by black Aspergillus isolated from Puerh and black tea can occur. An examination of 47 isolates from Puerh tea and black tea showed that none of these was A. niger. A part of the calmodulin gene in 17 isolates were sequenced, and these 17 isolates were all identified as Aspergillus acidus (=A. foetidus var. acidus). The rest of the 47 isolates were also identified as A. acidus from their metabolite profile. Neither production of ochratoxin A nor fumonisins B(2) and B(4) by any of the 47 isolates were observed.

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isolation of \textit{Aspergillus} series of black tea; Molecular Diagnostics 5 isolates of \textit{Aspergillus} \rightarrow \textit{Aspergillus acidus} (oral communication Ing. Savická)
Biogenic amines

- They are described as low molecular weight organic bases with aliphatic, aromatic, and heterocyclic structures.
- Biogenic amines have been reported in a variety of foods, such as fish, meat, cheese, vegetables, and wines.
- The most common biogenic amines found in foods are histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine, and agmatine.
- In addition, octopamine and dopamine have been found in meat and meat products and fish.
- The formation of biogenic amines in food by the microbial decarboxylation of amino acids can result in consumers suffering allergic reactions, characterized by difficulty in breathing, itching, rash, vomiting, fever, and hypertension.
- Traditionally, biogenic amine formation in food has been prevented, primarily by limiting microbial growth through chilling and freezing.
## Biogenic amines in fruits and vegetables

<table>
<thead>
<tr>
<th>Amin</th>
<th>Avocado</th>
<th>Banana skin</th>
<th>Orange</th>
<th>Tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamin</td>
<td>0,4-0,5</td>
<td>66-70</td>
<td>0,1</td>
<td></td>
</tr>
<tr>
<td>Epinefrin</td>
<td>0,25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinefrin</td>
<td></td>
<td>10,8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>1,0</td>
<td>2,5-8,0</td>
<td>1,2</td>
<td></td>
</tr>
<tr>
<td>Tyramin</td>
<td>2,3</td>
<td>6,5-9,4</td>
<td>1,0</td>
<td>0,4</td>
</tr>
</tbody>
</table>

Obsah v mg/100g potraviny.
Histamine

- **histamine poisoning** (scombroid poisoning) is a worldwide problem that occurs after the consumption of food containing biogenic amines, particularly histamine at concentrations higher than 500 ppm
- EC 2073/2005: fish: 200 a 400 mg/kg, HPLC test
- histamine poisoning manifests itself as an allergen-type reaction characterized by difficulty in breathing, itching, rash, vomiting, fever, and hypertension
- people having deficient natural mechanisms for detoxifying biogenic amines through genetic reasons or through inhibition due to the intake of antidepression medicines, such as monoamine oxidase inhibitors (MAOIs) are more susceptible to histamine poisoning
- histamine alone may not cause toxicity at a low level, but the presence of other biogenic amines such as **putrescine** and **cadaverine**, at concentrations 5 times higher than histamine, enhance the toxicity of histamine through the inhibition of histamine oxidizing enzymes
- oral toxicity levels for **putrescine, spermine, and spermidine** are 2000, 600, and 600 ppm, respectively
- tyramine alone at high levels can cause an intoxication known as the cheese reaction, which has similar symptoms to histamine poisoning
- conversion of histidine to histamine by histidine decarboxylase
- it is a substance arising from the amino acid histidine. It occurs mainly in certain white blood cells, but also in other organs. Its excessive release in the allergic reaction causing bronchoconstriction (asthma), urticaria, etc.
- although histamine is small compared to other biological molecules (containing only 17 atoms), it plays an important role in the body
- it is known to be involved in 23 different physiological functions, because of its chemical properties that allow it to be so versatile in binding
- physiologically histamine is very effective. It acts on smooth muscle, causing intense contractions of the uterus, dilates blood vessels and lowers the blood pressure
- histamine is also applied in the development of inflammation and also increases the secretion of gastric juice. The suppression of his work is part of the treatment of allergic conditions (H1 antihistamine) and stomach (H2 antihistamines)
Histamine

- histamine occurs **naturally in the human body**, while in a healthy person, there is a **regulatory mechanism that is capable of using enzymes toxic effects of histamine handle**
- the highest risk of excessive amounts of histamine is from **scombroid fish**, which include mackerel, tuna, herring or anchovies. It is because these fish have naturally higher levels of the amino acid histidine, of which histamine is - at a high microbial contamination of fish and, if not cold chain, especially in the warm season
- frequently histamine formed immediately after capture of fish, which then are not adequately cooled to a temperature of about +1°C
- another risk is the thermal processing operations, especially smoked fish, such as mackerel. Improper storage and with high microbial contamination (pollution) fish can create toxic levels before fish sensory effect as faulty
- excessive histamine content in fish can cause a particular disease associated itching of the skin, headaches, low blood pressure and skin rash
- toxic reactions can occur in a healthy person **within half an hour after eating**
- for this reason, the European legislation sets a maximum amount of the substance in fish in which a high amount of histidine can occur. The limit is set at **200 mg/kg**. State Veterinary Administration of the Czech Republic (SVS ČR) course, compliance with this limit in fish regularly
Thank you for your attention😊

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