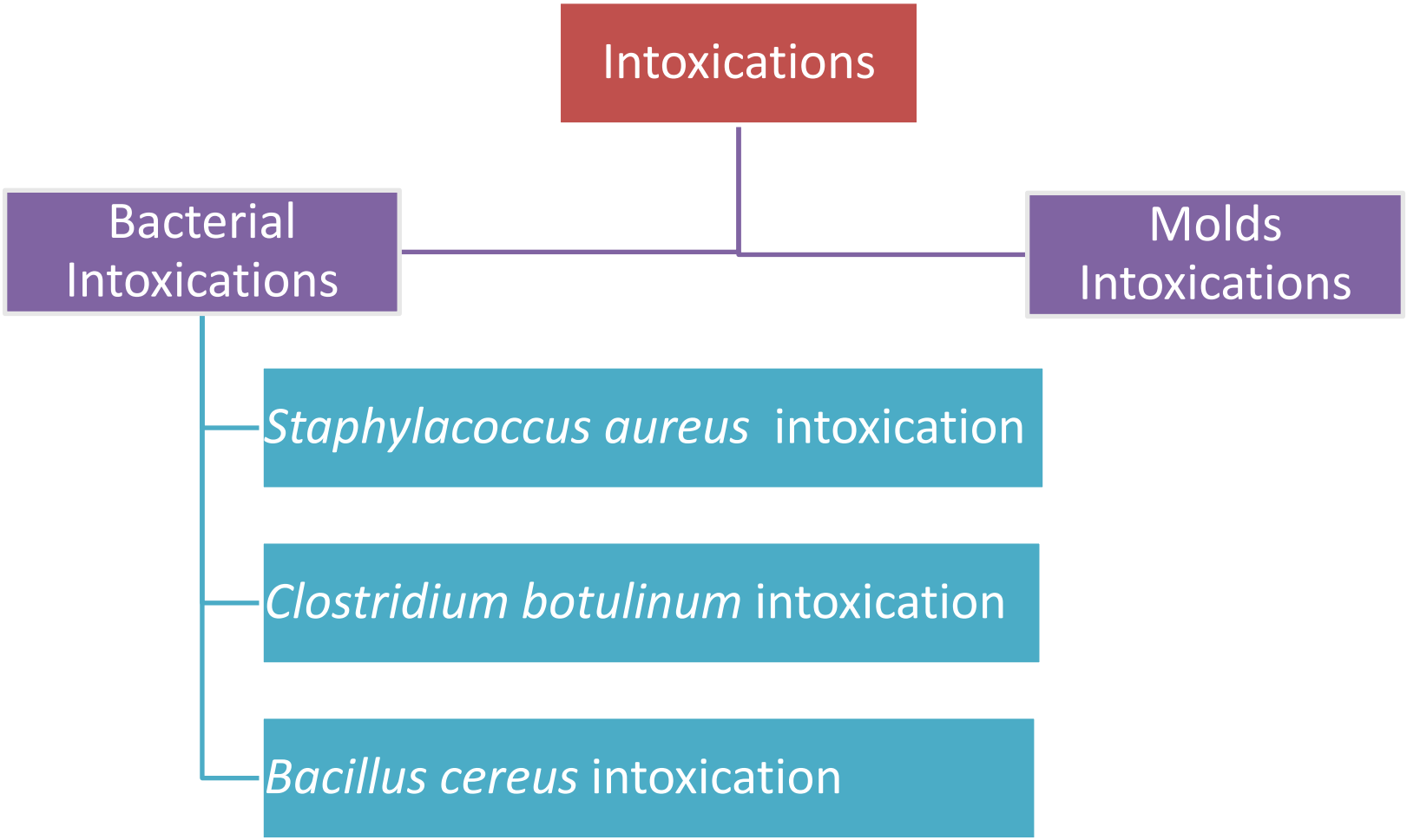
FE 305 FOOD MICROBIOLOGY
Intoxications and Toxico-infections

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Foodborne Bacterial Intoxications

- **Food intoxication is the illness caused by eating food that contains a toxin produced in the food by a microorganism.**
- As the definition states, it is not the microorganism but the toxin it has formed in the food that must be consumed.
- General features of intoxications:
  - the toxin is formed while the organism grows in the food;
  - the toxin may or may not be heat-resistant;
  - the toxin must be ingested with the food; live cells are not needed;
  - symptoms usually appear soon after the meal (30 minutes to 6 hours), except in botulism (12–36 hours);
  - the symptoms depend on the type of toxin;
  - fever is usually absent.

Foodborne Intoxications



Staphylococcus aureus Intoxication

- *Staphylococci* are Gram-positive cocci that form grape-like clusters under the microscope. *S. aureus* grows in many foods.
- Most enterotoxin-producing strains are coagulase-positive, but not all coagulase-positive *Staphylococci* produce enterotoxin.
- It grows between about 7 °C and 48 °C, with an optimum of 35–37 °C; enterotoxin is produced between about 10 °C and 46 °C.
- It tolerates low water activity (minimum a_{vv} 0.86, or 0.83 aerobically) and high salt concentrations (up to 15–20% NaCl).
- It is acid-tolerant and can grow down to about pH 4.5 under aerobic conditions.
- More than 20 staphylococcal enterotoxins are now known. The classical ones are SEA, SEB, SEC (C1, C2, C3), SED and SEE. Less than 1 µg of enterotoxin (about 100–200 ng) is enough to cause illness; the conditions for growth and toxin production differ from food to food.
- The enterotoxins are simple proteins. SEA, followed by SED, is the type most often involved in outbreaks. Their most important property is heat resistance: they survive boiling and normal cooking, and are only partly inactivated by retort temperatures.

Staphylococcus aureus Intoxication

- On solid media *Staphylococci* form colonies of various colours; the old species names were based on these colours:
  - golden-yellow pigment: *Staphylococcus aureus*;
  - lemon-yellow pigment: "*S. citreus*" (a name no longer used);
  - white pigment: "*S. albus*", now *S. epidermidis*.
- Enterotoxigenic *S. aureus* strains typically show β-haemolysis on blood agar, coagulate human or rabbit plasma (coagulase-positive), grow in 7.5% NaCl and ferment mannitol (Baird-Parker and mannitol salt agar are used for their isolation).
- Illness begins 1–6 hours (usually 2–4 hours) after the contaminated food is eaten.
- Symptoms are nausea, violent vomiting, abdominal cramps and diarrhoea.
- The symptoms may be mild, moderate or severe and usually disappear within one or two days.
- Recovery is normally complete; deaths are rare, and most cases need no treatment beyond fluids.

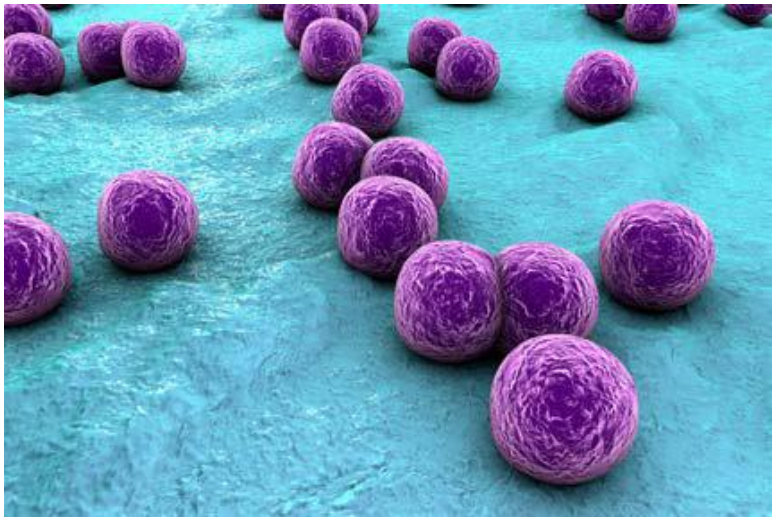
Staphylococcus aureus Intoxication

- Humans are the main reservoir of *S. aureus*:
  - on the skin and in the mouth, throat and nose;
  - about 30–50% of healthy people carry *S. aureus*;
  - infected wounds, cuts, sinuses and pimples are full of it.
  - It is also found in air, soil, water, sewage, on plant surfaces and in meat, poultry and dairy products.
- Foods involved in outbreaks are mainly protein-rich foods that are handled after cooking:
  - meat and canned beef, salami, bacon, sausages, salads, meat-filled sandwiches, cheese, puddings, ice cream and cream-filled bakery products.

Staphylococcus aureus

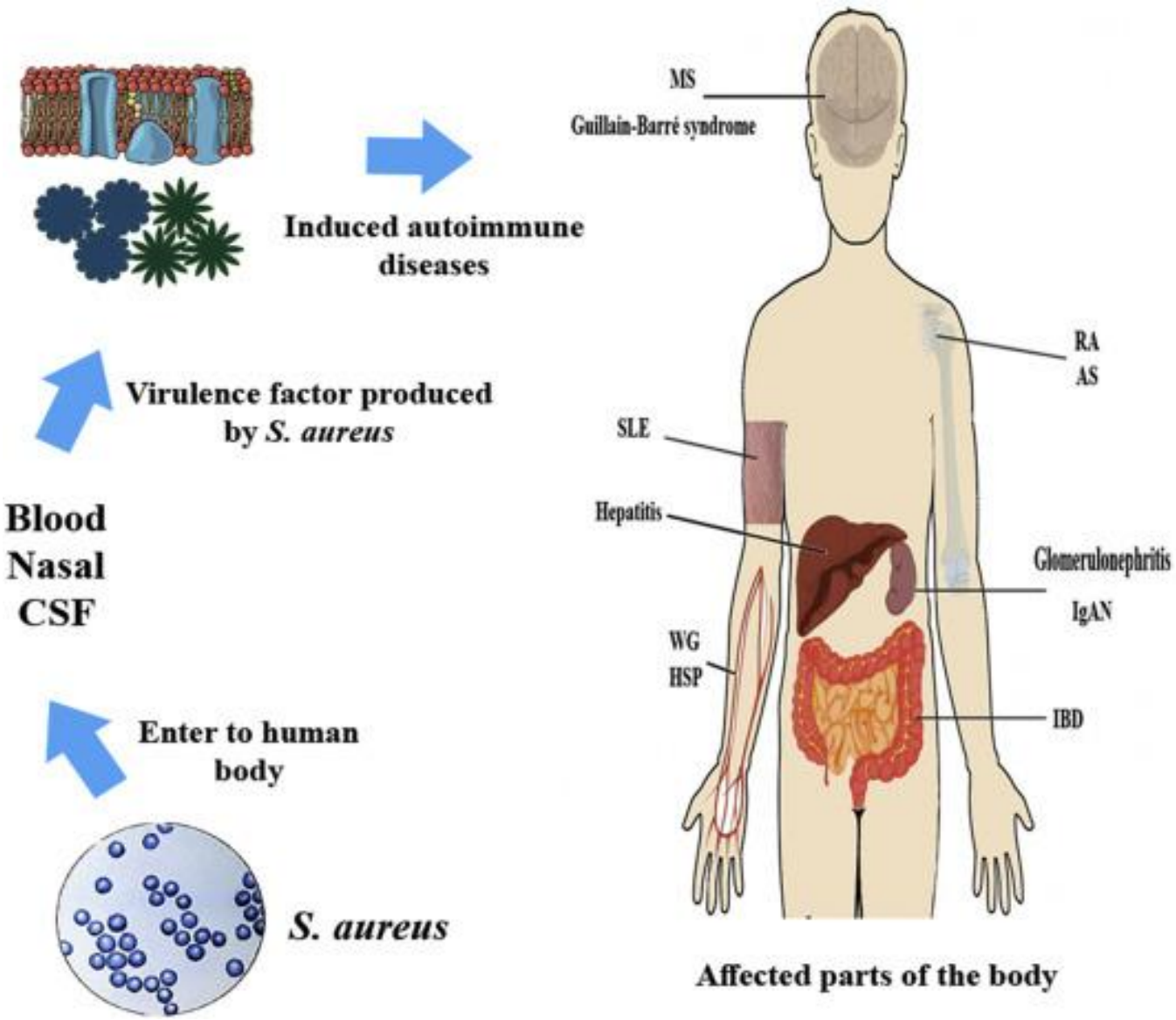


a) Unstained



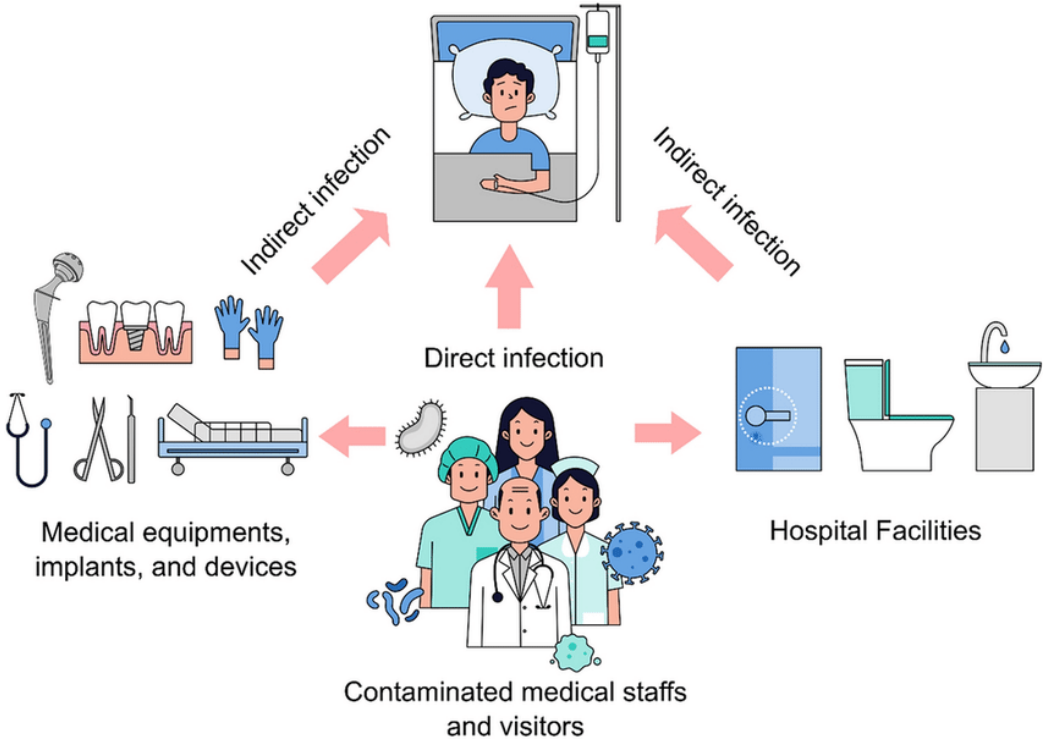
b) Gram-stained

S. aureus Intoxication Mechanism



Staphylococcus aureus Intoxication

- This type of food poisoning occurs:



- Healthy people carry these bacteria in the mouth, throat and nose. Infected wounds are particularly rich in staphylococci, as are any inflamed parts of the body: mouth, throat, sinuses, ears, nose and acne.

Staphylococcal Food Poisoning: A Classic Scenario

***S. aureus* in the food**

08:00	A cook with a staphylococcal skin infection fills cream pastries by hand	10^2 cells/g
09:00 - 16:00	The pastries stand in a warm kitchen at 25 - 30 °C	10^6 cells/g
at 10^5 cells/g	Enterotoxin is formed in the cream - less than 1 µg is enough	toxin present
17:00	The pastries are warmed in the oven before serving	cells killed, toxin intact
19:00 - 21:00	Guests eat; 1 - 6 h later: sudden nausea, violent vomiting, cramps	no fever

What the laboratory finds

enterotoxin detected in the leftovers, but few or no live *S. aureus* - the heating killed the cells

What would have prevented it

no work with infected skin; covered cuts; hand hygiene; keep cream fillings below 5 °C and serve within 2 h

Staphylococcus aureus Intoxication

- The bacteria reach food through careless workers: coughing or sneezing over food, touching the nose, mouth, ears, door handles, money or tissues and then handling food, or continuing to prepare food without washing the hands properly after using the toilet.
- Protein-rich foods and dishes made with them are the most suitable for staphylococcal poisoning, and the way they are prepared often allows the bacteria to multiply rapidly.
  - Examples are meat, chicken, fish, milk, eggs, shellfish, salami, sausages, minced-meat dishes, soups and sauces made with broth, egg–sugar–milk mixtures and cream cakes.
- Because these foods are of low acidity, staphylococci multiply easily in them. When an acid food such as yoghurt is mixed with eggs, its acidity decreases and it can also become suitable for staphylococci.
- Foods that are handled a great deal during preparation - deboning, chopping, mincing, kneading and shaping - are the most likely to be contaminated. *S. aureus* competes poorly with other bacteria, so it is most dangerous in cooked foods that are contaminated afterwards.

Staphylococcus aureus Intoxication

- *S. aureus* grows between about 7 °C and 48 °C; the cells are killed by pasteurisation and normal cooking.
- Keep hot food hot (60 °C and above) and cold food cold (5 °C and below). Cooked food that will not be served immediately, and leftovers, should be cooled rapidly - for example by placing the container in cold water and stirring, if this does not spoil the food.
- Cooked foods and perishable meat should not be kept at room temperature for more than two hours; this prevents the bacteria from multiplying and forming toxin.
- The enterotoxins are heat-resistant and are not easily destroyed. Boiling for 20–60 minutes does not inactivate them completely. Cooking or reheating kills the staphylococci but not the toxin, so poisoning can still occur.
- Food handlers must be healthy, must cover cuts and wounds, and must follow the rules of personal hygiene at work.

Clostridium botulinum Intoxication (Botulism)

- Botulism is caused by the neurotoxin of *Clostridium botulinum* and occurs in three main forms.
  - The most common is foodborne botulism, caused by eating food in which the toxin has already been formed.
  - Wound botulism is rare and occurs when *C. botulinum* produces its toxin in an infected wound.
  - In infant botulism, *C. botulinum* colonises the intestine of babies under one year and produces its toxin there; honey is a known source of spores. In wound and infant botulism the toxin is formed in vivo after infection (toxicoinfection).
- The disease and its agent were first described by Émile van Ermengem after a ham-associated outbreak in Belgium in 1895. The illness results from ingesting the exotoxin of *Clostridium botulinum* with food.
- Botulism is more common in some parts of Europe and in North America than in Türkiye, where well-cooked dishes predominate; cases reported in Türkiye are mostly linked to home-prepared foods.
  - Botulism is usually caused by improperly prepared canned foods, sausages, cured meats and fermented fish, especially when they are made at home.

Clostridium botulinum Intoxication (Botulism)

- Although botulism is less common than other foodborne diseases, it is important because of its high fatality rate.
  - In 766 botulism outbreaks recorded in the USA between 1899 and 1977, 1 966 people were affected and 999 of them (50.8%) died.
  - Between 1960 and 1977, 448 people were affected in 186 US outbreaks and 82 (18.3%) died;
  - Between 1978 and 1983, 201 people were affected in 91 outbreaks and 26 (12.9%) died. With antitoxin and intensive care, the fatality rate today is about 5–10%.
- Low-acid canned vegetables, fish and meat products and smoked meat and fish are the foods most often involved, but botulism has also been caused by unusual foods such as baked potatoes wrapped in aluminium foil and garlic stored in oil.
  - In 1978, 59 people contracted type A botulism from potatoes baked in foil and kept at room temperature,
  - In 1985, 36 people contracted type B botulism from chopped garlic kept in oil.

Clostridium botulinum Intoxication (Botulism)

- *C. botulinum* is an obligately anaerobic, Gram-positive, non-capsulated rod (0.5–0.8 × 3.0–8.0 μm), motile by peritrichous flagella, that forms oval spores located terminally or subterminally in the cell.
- It is divided into seven classical types according to the antigenic structure of its neurotoxins: A, B, C, D, E, F and G.
- Type A toxin is the most potent, followed by types B and F. Types A, B and E (and occasionally F) cause botulism in humans.
- The general characteristics of the types are:
  - Type A: the main cause of human botulism in the western USA; more toxic than type B.
  - Type B: common worldwide, especially in Europe and the eastern USA; a human pathogen.
  - Type C: causes botulism in poultry, wild ducks, cattle and other mammals.
  - Type D: pathogenic for cattle and other animals, especially in South Africa; not known to cause disease in humans.
  - Type E: lives in the sediments of streams, lakes and seas; a human pathogen important in fish and seafood.
  - Type F: similar to types A and B in toxin properties and a cause of human botulism; first isolated in Denmark.
  - Type G: isolated from soil in Argentina; now classified as a separate species, *C. argentinense*. It has not been proven to cause foodborne botulism.

Clostridium botulinum Intoxication (Botulism)

- According to their cultural and physiological characteristics, the botulinum-toxin-producing clostridia are divided into four groups:
  - Group I (proteolytic): all type A strains and the proteolytic strains of types B and F.
  - Group II (non-proteolytic): all type E strains and the non-proteolytic strains of types B and F.
  - Group III: types C and D, both non-proteolytic (animal botulism).
  - Group IV: type G (*C. argentinense*), weakly proteolytic. Some strains of *C. butyricum* (type E) and *C. baratii* (type F) also produce botulinum toxin.
- The growth and toxin production of *C. botulinum* in foods depend on several factors:
  - the type and characteristics of the food (nutrients, water content and a_{vv}, pH, redox potential, salt content, etc.);
  - the storage temperature and time.
- *C. botulinum* grows best at neutral pH. Group I strains do not grow or produce toxin at pH 4.6 or below (Group II at pH 5.0 or below); the maximum pH for growth is about 8.9.
- For this reason, foods with a pH of 4.6 or below (acid foods) are regarded as safe with respect to botulism.
- Nevertheless, botulism has been caused by some foods with a pH below 4.6, such as tomato juice and fruit preserves.

Group I and Group II *Clostridium botulinum*

	Group I - proteolytic	Group II - non-proteolytic
Toxin types	A, B, F	E, B, F
Minimum temperature	10 - 12 °C	3.3 °C - grows in the refrigerator
Minimum pH	4.6	5.0
Minimum aw	0.94 (about 10% NaCl)	0.97 (about 5% NaCl)
Spore heat resistance	high: $D_{121} \approx 0.2$ min	low: 90 °C 10 min = 6 log
Typical foods	low-acid canned and bottled foods	vacuum-packed chilled fish and meat
Key control	botulinum cook $F_o \geq 3$ min	chill ≤ 3 °C or limit shelf life

The "10-day rule" for chilled vacuum and MAP foods

at 3 - 8 °C, shelf life ≤ 10 days unless another hurdle (90 °C 10 min, pH ≤ 5.0, aw ≤ 0.97, salt ≥ 3.5%) controls Group II

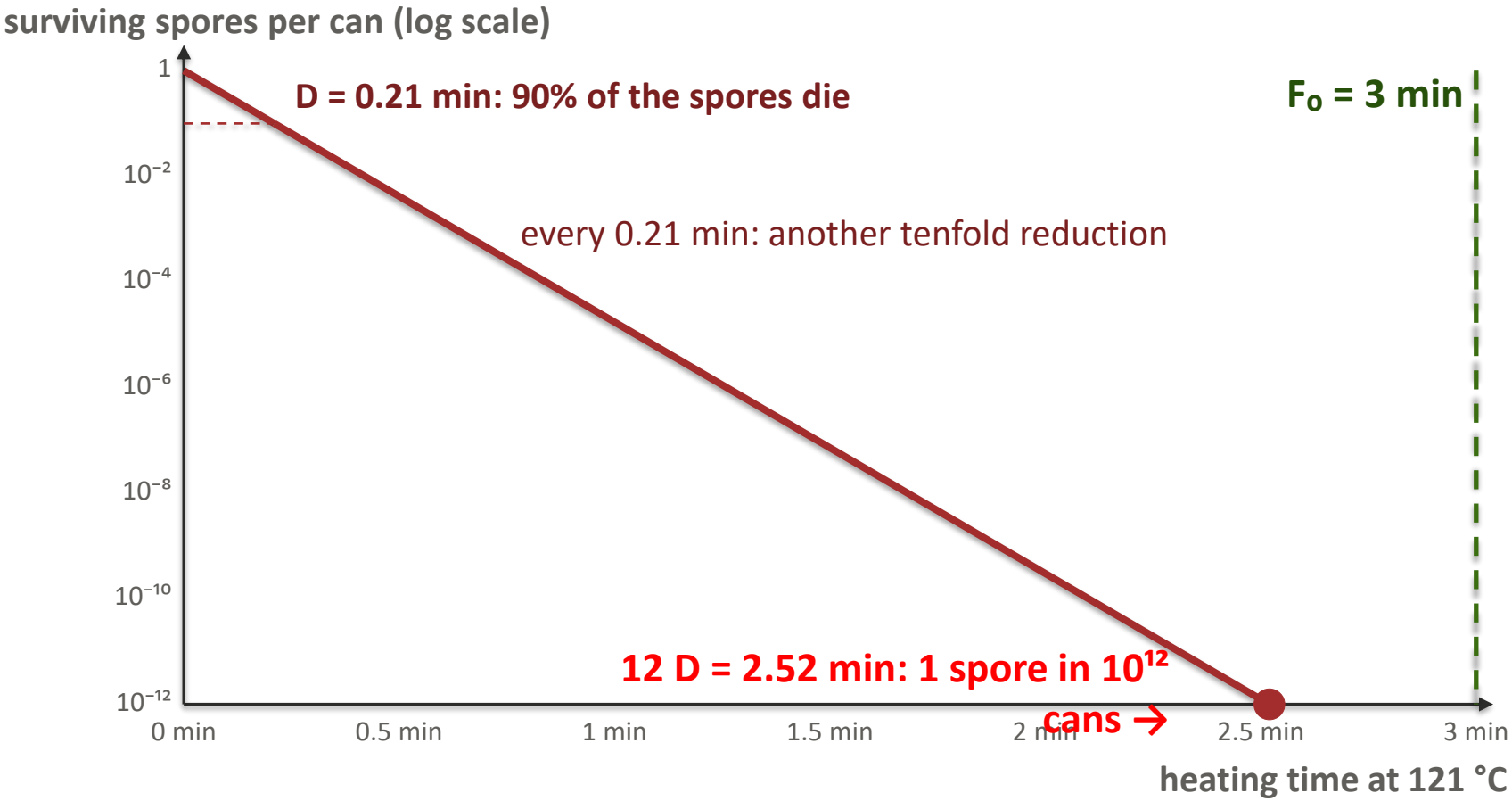
Clostridium botulinum Intoxication (Botulism)

- The conditions that allow *C. botulinum* to grow in such foods are:
  - the growth of other microorganisms (moulds such as *Penicillium* and *Aspergillus*, film yeasts, *Trichosporon* and some *Bacillus* species) that consume acid and raise the pH of a normally acid food to a level at which *C. botulinum* can grow;
  - uneven acidification of foods whose pH is lowered by adding acid (e.g. GDL), leaving regions of higher pH.
- Meat, fish and low- and medium-acid canned foods are favourable for toxin production. The minimum a_{vv} for growth is 0.94 for Group I (types A, proteolytic B and F) and 0.97 for Group II (type E, non-proteolytic B and F).
- Group I grows between 10 and 48 °C (optimum 35–40 °C); Group II grows between 3.3 and about 45 °C (optimum 25–30 °C) and can therefore grow in chilled foods.
- The neurotoxin is a protein of about 150 kDa; in foods it occurs as a complex with other proteins (300–900 kDa) that protects it in the stomach.
- Botulinum neurotoxin is the most potent biological toxin known: its lethal dose for humans is estimated at about 1 ng per kg body weight, so 1 gram could in theory kill millions of people.

Clostridium botulinum Intoxication (Botulism)

- An important feature of botulinum toxin is that, unlike the spores, it is heat-sensitive. The toxin is formed best at about 35 °C.
  - The toxin is inactivated by heating at 80 °C for 10 minutes or at 85 °C for 5 minutes;
  - boiling for 10 minutes destroys it completely.
  - Spores of Group I are highly heat-resistant ($D_{121} \approx 0.2$ min); the "botulinum cook" ($F_0 = 3$ min at 121 °C) reduces them by 12 log units.
  - Spores of Group II (type E) are much less resistant; heating at 90 °C for 10 minutes gives a 6-log reduction.
  - The toxin is inactivated by sunlight and air, but in the dark and without air it remains stable even at −79 °C. It is also acid-resistant and survives passage through the stomach.
- Poisoning is usually caused by low-acid canned meat and vegetables, smoked sausage, raw ham and salted or pickled fish containing less than 5% salt.
- The spores germinate where oxygen is absent. Non-proteolytic strains release the toxin in a less active form, which is activated ("nicked") by proteolytic enzymes such as trypsin in the intestine.
- The toxin blocks the release of acetylcholine at the junction between nerve and muscle, so that nerve impulses no longer cause muscle contraction (flaccid paralysis). Because the toxin breaks down in 10 minutes of boiling, thorough heating of suspect food prevents intoxication.

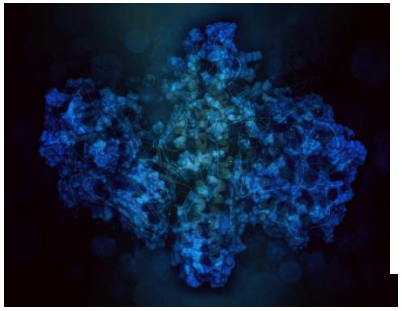
The Botulinum Cook: the 12-D Concept



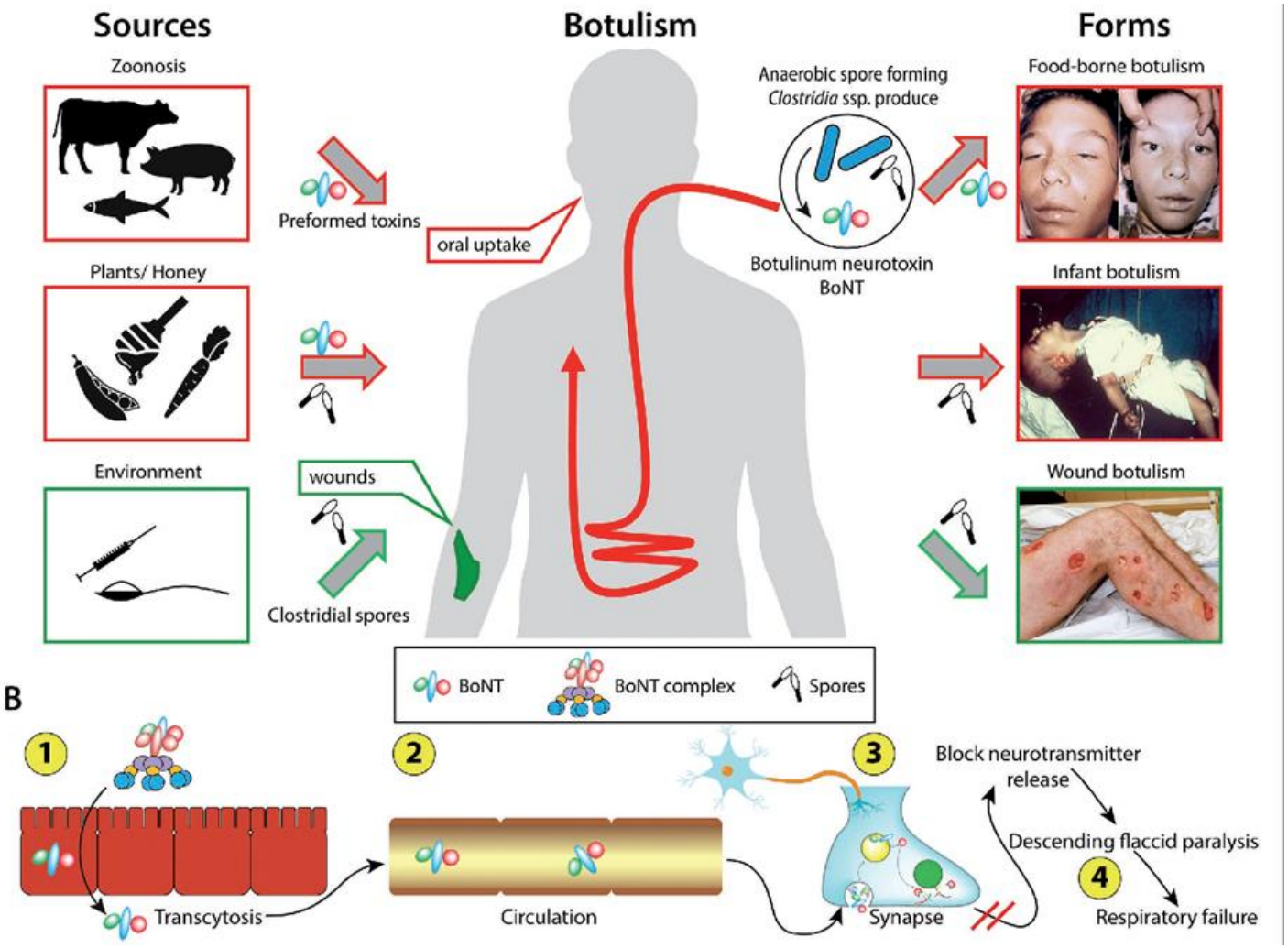
Low-acid foods (pH > 4.6)
need the full botulinum cook,
$F_o \geq 3$ min in the coldest point of the can

Acid foods (pH ≤ 4.6)
C. botulinum cannot grow - a much milder process (below 100 °C) is enough

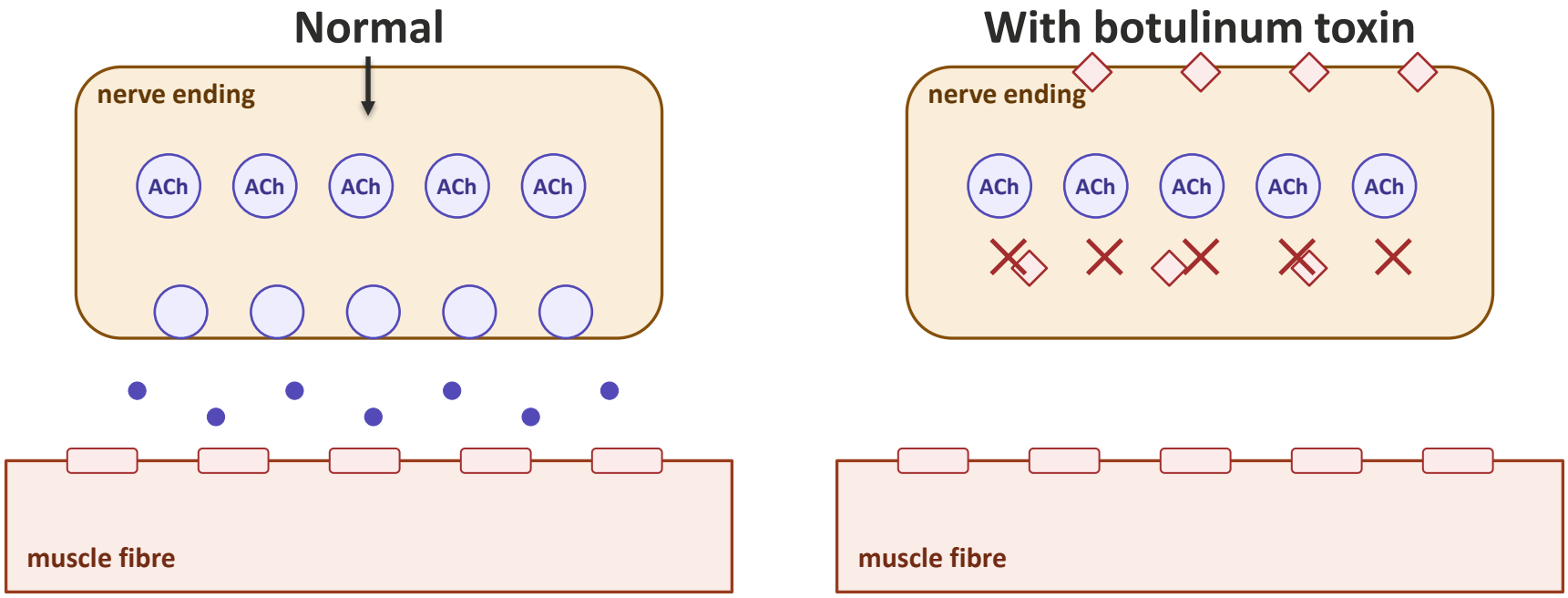
Mechanism of Foodborne Botulism



C. botulinum toxins



Botulinum Toxin at the Nerve–Muscle Junction



1 impulse arrives; vesicles full of acetylcholine
2 SNARE proteins fuse vesicles; ACh released
3 receptors bind ACh - the muscle contracts

1 toxin binds to the nerve ending
2 light chain enters and cuts the SNARE proteins
3 no ACh release - flaccid paralysis

Consequences for treatment

antitoxin neutralises only toxin still in the blood - give it early; recovery needs new nerve endings (weeks to months)

Clostridium botulinum Intoxication (Botulism)

- Low-acid home-canned foods are the leading cause of botulism; one US study attributed 72% of cases to home-canned foods.
  - In Europe, canned and cured meat products are the most common vehicles;
  - In the USA, low-acid canned vegetables (maize, beans, spinach, asparagus, beets);
  - In Canada, Japan, the former Soviet countries and Iran, fish and fish products.
- The incubation period of foodborne botulism is usually 12–36 hours, but it can range from 2 hours to 8 days.
- The shorter the incubation period, the higher the fatality rate. Early symptoms may include nausea, vomiting, diarrhoea, weakness, dizziness and headache; constipation follows once the typical neurological signs begin.
- Botulism begins with muscle weakness: drooping eyelids, dilated pupils (mydriasis), blurred and double vision (diplopia), dry mouth, difficulty in speaking and swallowing, and weakness of the facial, tongue and limb muscles.
- A descending, symmetrical paralysis follows; breathing becomes irregular, and in severe cases death occurs within 3–5 days from paralysis of the respiratory muscles. Early mechanical ventilation can save the patient's life.

Clostridium botulinum Intoxication (Botulism)

- Death may occur at any time between 24 hours and 2–3 weeks.
- Patients who survive the first 10 days usually recover.
- Historically the fatality rate was 30–80%, depending on the toxin type and region; with antitoxin and intensive care it is now about 5–10%.
- Full recovery takes months (6 months or more), and partial paralysis may persist for 6–8 months while new nerve endings grow.
- The specific treatment is neutralisation of the circulating toxin with antitoxin. A heptavalent antitoxin (types A–G) is now used; if the toxin type is known, a monovalent serum of that type may be given.
- Antitoxin cannot reverse paralysis that has already developed, so it must be given as early as possible. Removal of toxin from the gut and prolonged mechanical ventilation, if necessary through a tracheostomy, are the other main measures.

Clostridium botulinum Intoxication (Botulism)

- Points for the food industry:
  - The most important is the exact application of the validated time–temperature processes prescribed for canned foods ($F_0 \geq 3$ min for low-acid foods).
  - All foods with partly or completely anaerobic regions that could allow *C. botulinum* to grow must be protected by pH, salt, a_{vv}, chemical preservatives (such as sodium nitrite in meat products), refrigeration and other antimicrobial hurdles.
- Points for the consumer:
  - Never use cans that are swollen, rusty, damaged or leaking, or whose contents spurt, foam, are discoloured or smell bad when opened. Such foods must not even be tasted; they must be destroyed safely.
  - Do not eat cooked food that has been kept at room temperature without reheating.
  - Do not keep raw or cooked foods at room temperature after thawing, and discard forgotten leftovers.
  - Store canned foods in a cool, dry place, and do not give honey to infants under one year.

Bacillus cereus Intoxication

- *Bacillus cereus* belongs to the family Bacillaceae. It is a spore-forming, facultatively anaerobic rod (1.0–1.2 × 3.0–5.0 µm) with peritrichous flagella. Its optimum growth temperature is 28–35 °C, usually about 30 °C.
- It is a Gram-positive, motile, endospore-forming rod whose spores are relatively heat-resistant. It is widely distributed in soil, dust and water, and is found in meat, eggs, rice, pasta, vegetables and dairy products.
- *B. cereus* is very common in milk and is a thermoduric dairy bacterium: its spores survive pasteurisation (63 °C for 30 minutes). In pasteurised milk without post-pasteurisation contamination it is the most important organism limiting quality, causing sweet curdling and the defect known as bitty cream.
- Although it is common in milk, *B. cereus* poisoning from dairy products is rare, because milk containing high numbers of the organism is usually rejected as spoiled. Besides milk, it is often isolated from rice and other grains, starch, spices, dried foods, meat and chicken.
- *B. cereus* was first identified as a cause of food poisoning by Hauge in Norway (1950–1955), in four outbreaks affecting 600 people. In all four cases the food was a vanilla sauce prepared the day before and kept at room temperature. Outbreaks were later recognised in many European countries.

Bacillus cereus Intoxication

- A very wide range of foods can cause *B. cereus* food poisoning; starchy foods that are kept for some time before serving are especially susceptible.
- The high numbers of the organism found in implicated foods show that it has multiplied in the food. The emetic type is a true intoxication; the diarrhoeal type is a toxicoinfection in which the enterotoxins are formed in the small intestine.
- Toxin formation depends on the nutrients present; the enterotoxins are produced during the exponential phase of growth.
- The diarrhoeal enterotoxins are sensitive to trypsin and pepsin and are inactivated by them within 60 minutes at 37 °C. They are stable for 30 minutes at 45 °C but are inactivated within 5–30 minutes at 56 °C.
- The emetic toxin, cereulide, is a small cyclic peptide that is extremely resistant to heat (it survives 121 °C for 90 minutes), to pH 2–11 and to trypsin and pepsin. It is formed in the food during growth, especially when cooked rice or pasta is kept at room temperature.

Bacillus cereus Intoxication

- *B. cereus* causes two types of food poisoning:
  - Emetic type (intoxication): cereulide, a cyclic peptide, is preformed in the food. Incubation 0.5–6 hours (usually 2–5 hours); symptoms are nausea, vomiting and malaise, occasionally diarrhoea.
    - Foods: fried and cooked rice, pasta, pizza.
  - Diarrhoeal type (toxicoinfection): symptoms appear after 8–16 hours; ingested cells and spores survive the stomach, multiply in the small intestine and release enterotoxins.
    - The diarrhoeal enterotoxins are heat-labile proteins: haemolysin BL (Hbl), non-haemolytic enterotoxin (Nhe) and cytotoxin K (CytK).
    - Foods: cereal foods, especially maize and cornflour, mashed potatoes, vegetables, minced meat, some meat products, puddings and soups.
    - Symptoms: abdominal pain and watery diarrhoea, sometimes with nausea and vomiting.
- To prevent *B. cereus* poisoning, foods at risk should be prepared in small portions just before they are eaten and heated adequately. Food that is not eaten immediately should be cooled rapidly and kept chilled, and should be reheated above 74 °C. Remember that reheating does not destroy cereulide.

Two Faces of *Bacillus cereus*

	Emetic type intoxication	**Diarrhoeal type** toxicoinfection
Toxin	cereulide - a small cyclic peptide	Hbl, Nhe, CytK - proteins
Where it is made	in the food, before eating	in the small intestine
Heat stability	survives 121 °C for 90 min	destroyed at 56 °C
Incubation	0.5 - 6 hours	8 - 16 hours
Main symptoms	nausea, vomiting	abdominal pain, watery diarrhoea
Typical foods	fried and cooked rice, pasta	meat, vegetables, sauces, puddings
Resembles	*S. aureus* intoxication	*C. perfringens* poisoning

Control for both

cool cooked rice and pasta within 2 hours and keep below 5 °C or above 60 °C - reheating does not destroy cereulide

Foodborne Toxicoinfections

- A toxicoinfection is the disease that results when food containing live microorganisms is eaten and the organisms then produce their toxin inside the body.
- Features of foodborne toxicoinfections:
  - Large numbers of cells must usually be ingested (about 10^5–10^8). Spore-formers such as *C. perfringens* sporulate in the intestine and release their toxin when the cells lyse.
  - Gram-negative agents such as ETEC and *V. cholerae* also need a high dose, because most cells are killed by gastric acid; the survivors attach to the small intestine and secrete enterotoxin there.
  - The organisms do not invade the intestinal tissue; the damage is caused by the toxin.
    - Examples:
      - Enteropathogenic *Escherichia coli* (EPEC)
      - Enterotoxigenic *Escherichia coli* (ETEC)
      - *Clostridium perfringens*
      - *Bacillus cereus* (diarrhoeal type)
      - *Vibrio cholerae*
      - *Aeromonas hydrophila*

Pathogenic *Escherichia coli*

- *Escherichia coli* is normally a harmless commensal in the intestines of humans and warm-blooded animals, but some strains are pathogenic and cause diarrhoea. Six pathotypes are recognised; the four most important for food are EPEC, EIEC, ETEC and EHEC/STEC.
- EPEC: a cause of infant diarrhoea, mainly in developing countries. The diarrhoea is watery and contains mucus. The bacteria form attaching and effacing lesions on the small intestine.
- ETEC: the main cause of traveller's diarrhoea. It is transmitted by faecally contaminated water and by food washed with such water. The infective dose is high (about 10^8 cells). It produces a heat-labile toxin (LT) similar to cholera toxin and a heat-stable toxin (ST).
- EIEC: invades the colonic epithelium like *Shigella* and causes dysentery. The infective dose is about 10^6 cells.
- EHEC/STEC: the best-known serotype is O157:H7. It causes haemorrhagic colitis, haemolytic uraemic syndrome (HUS), especially in children, and thrombotic thrombocytopenic purpura (TTP), mainly in adults. The infective dose is below 100 cells.

Pathogenic *Escherichia coli*

- In haemorrhagic colitis, sudden abdominal cramps are followed within 24 hours by watery diarrhoea, which then becomes grossly bloody. Vomiting may occur; fever is absent or slight. The illness usually lasts 2–9 days.
- Haemolytic uraemic syndrome (HUS) is the leading cause of acute kidney failure in children. Shiga toxin damages the endothelial cells of small blood vessels, small clots form and block the vessels of the kidneys and other organs, and waste products accumulate in the blood. Patients may need blood transfusion and dialysis; heart failure, seizures and coma can occur.
- Thrombotic thrombocytopenic purpura (TTP) resembles HUS but mainly affects the nervous system. It is usually seen in adults and causes anaemia, fluctuating neurological symptoms, fever and mild kidney impairment; clots in the brain make it frequently fatal.

Enterotoxigenic *Escherichia coli* (ETEC)

- Unlike EIEC and STEC, ETEC does not invade or damage the intestinal wall: the cells attach to the small intestine after ingestion, multiply and secrete enterotoxins. ETEC is the leading cause of traveller's diarrhoea (“tourist diarrhoea”), and its symptoms resemble mild cholera.

Properties

- ETEC is a Gram-negative, non-spore-forming, motile, facultatively anaerobic short rod. Its optimum growth temperature is 30–37 °C, and it is destroyed by pasteurisation.

Sources

- Water and food contaminated with ETEC cause the disease. Vegetables, salads, potatoes, cheese, fish, meat and meat products are risky foods. ETEC reaches water and food through faeces as a result of poor hygiene; inadequate cooking and improper storage are also important causes.

Enterotoxigenic *Escherichia coli* (ETEC)

Symptoms

- Symptoms begin 10–72 hours (usually 1–3 days) after the food is eaten.
- Profuse watery diarrhoea is the main symptom, sometimes with vomiting and cramps.
- The illness lasts 3–4 days. In severe cases, dehydration and shock may occur.

Prevention

- To prevent ETEC infection:
  - observe the rules of hygiene and sanitation - this is the most important rule;
  - cook food thoroughly and store cooked food at safe temperatures; when travelling, drink only treated water.

Clostridium perfringens

The illness caused by *C. perfringens* in food is called perfringens food poisoning. Its symptoms are mild and short, and because patients rarely see a doctor, the number of recorded cases is much lower than the true number.

Properties

C. perfringens is a Gram-positive, anaerobic, non-motile, spore-forming rod. Its optimum growth temperature is 43–45 °C; about 37 °C is optimal for sporulation and toxin production. It cannot grow at refrigeration temperature. The vegetative cells are heat-sensitive and are killed by pasteurisation, but the spores are heat-resistant and survive for hours at 100 °C.

Sources

C. perfringens lives in the intestines of humans and animals and reaches food through sewage and faeces. Protein foods are involved: meat and meat products, gravies and soups made with broth, meat pies and salads containing meat. Cooking drives out oxygen, so this anaerobe can multiply in the cooked food.

Poisoning usually follows meals cooked one or two days earlier, cooled slowly and reheated inadequately. Foods produced in large quantities and left to cool slowly, and foods kept warm until serving, are especially risky.

Clostridium perfringens

Symptoms

- Symptoms begin 8–22 hours (usually about 12 hours) after the food is eaten: abdominal cramps, diarrhoea and fatigue. The illness ends by itself within 24 hours and is mild, so a doctor is rarely consulted.

Prevention

- To prevent perfringens poisoning:
  - cook food thoroughly and serve it without delay;
  - if food is not eaten immediately, divide it into small portions and cool it rapidly in the refrigerator;
  - reheat refrigerated food thoroughly (above 74 °C) before eating, and observe the rules of hygiene.

Bacillus cereus

Because most countries do not report *B. cereus* food poisoning systematically, data on the disease are limited. The emetic form is also called “fried rice syndrome”, because it is usually caused by rice dishes left at room temperature.

Properties

- *B. cereus* is a Gram-positive, spore-forming, motile, facultatively anaerobic rod. Its optimum growth temperature is 30–40 °C. The vegetative cells are killed by pasteurisation, but the spores survive high temperatures.

Sources

- *B. cereus* is a soil organism and is therefore common on vegetables and fruit. Salads, meat, milk and dairy products, sauces, cereals, legumes, pasta and rice are risky foods.

Symptoms

- In the diarrhoeal form, symptoms appear 8–16 hours after the food is eaten; in the emetic form, after 0.5–6 hours. Recovery is rapid. The diarrhoeal form resembles perfringens poisoning.

Prevention

- To prevent *B. cereus* poisoning:
  - store cereals and dried legumes in cool, dry conditions;
  - cook rice dishes in the quantities needed for the day, and do not reheat them more than once;
  - cook food thoroughly;
  - keep cooked dishes either in the refrigerator or above 60 °C;
  - some psychrotrophic strains of the *B. cereus* group can grow slowly at refrigeration temperature, so do not keep food in the refrigerator for too long;
  - observe personal and kitchen hygiene.

Bacillus cereus

Foodborne outbreaks

- *B. cereus* causes two types of food poisoning:
  - the emetic type and the diarrhoeal type.
- The emetic toxin, preformed in the food during growth, causes vomiting.
- **Living cells or spores that produce enterotoxins in the small intestine cause the diarrhoeal illness.**
  - Large numbers of cells or spores (10^5–10^8 per gram) must be ingested with food to cause illness.
  - Diarrhoeal symptoms appear after 8–16 hours (mostly about 12 hours).
  - Symptoms are abdominal pain and watery diarrhoea, sometimes with nausea and vomiting.
  - Three enterotoxins can cause the diarrhoeal illness: haemolysin BL (lyses red blood cells), non-haemolytic enterotoxin (Nhe) and cytotoxin K.

Vibrio cholerae

V. cholerae causes cholera, which can spread in epidemics with high mortality. Since 1817 there have been seven cholera pandemics; the seventh, caused by the El Tor biotype, began in 1961 and continues today.

Properties

- *V. cholerae* is a Gram-negative, non-spore-forming, motile, facultatively anaerobic, comma-shaped rod.
- It is sensitive to heat but tolerates cold. Its optimum growth temperature is 30–37 °C. It cannot grow in acid foods, but alkaline foods are very suitable for it. It is killed by pasteurisation, and it survives at 5–10 °C in cooked foods.

Sources

- Like all *Vibrio* species, *V. cholerae* is associated with seawater and seafood, but cholera is spread mainly by food and water contaminated with the faeces of patients. Undercooked fish, oysters, crabs and shrimps are risky foods.

Vibrio cholerae

Sources

- *V. cholerae* is widely distributed in aquatic environments.
- The foods most often associated with cholera are crabs, shrimps, raw fish, oysters, clams, rice, frozen coconut milk and raw fruits and vegetables.

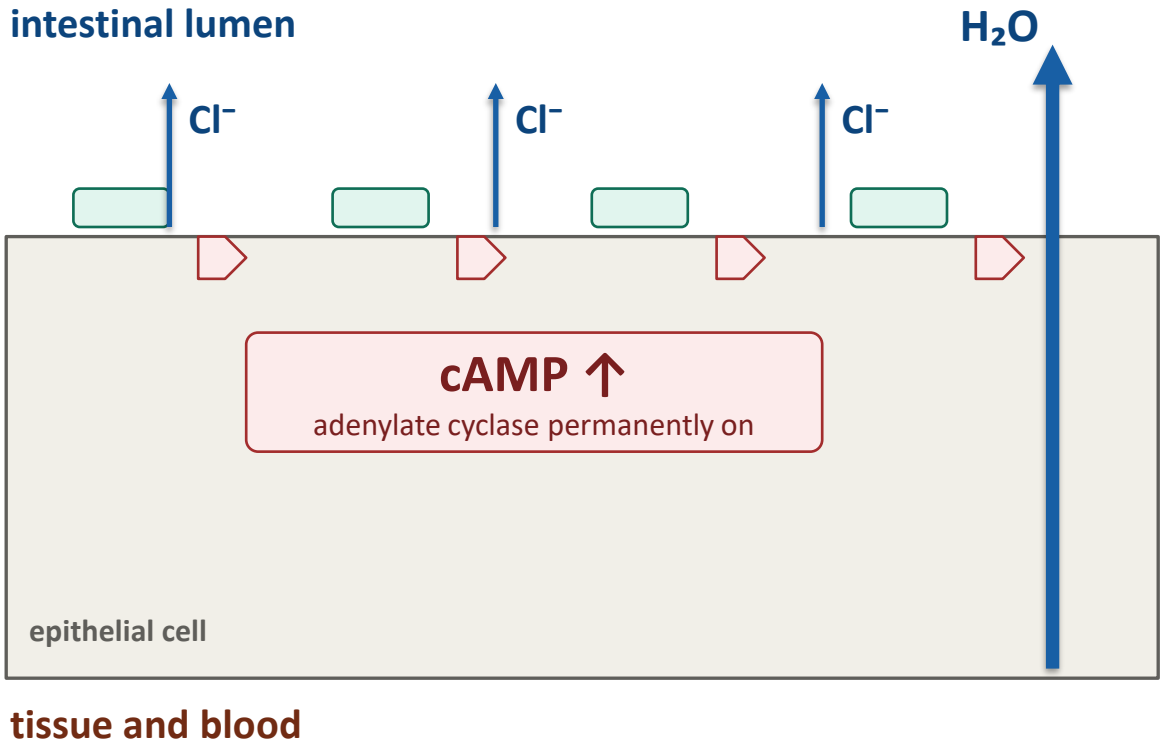
Symptoms

- Symptoms appear from 12 hours to 5 days after contaminated water or food is consumed. The main symptom is profuse watery diarrhoea, together with vomiting, weakness, muscle cramps and confusion.
- Most infected people have no or only mild symptoms, but they shed the bacteria in their faeces and can infect others.

Prevention

- To prevent cholera:
  - follow the rules of hygiene;
  - since most epidemics are caused by water, ensure safe, treated drinking water;
  - cook food thoroughly;
  - do not eat raw seafood;
  - keep food refrigerated; freezing also reduces the number of *V. cholerae* in food. Oral cholera vaccines are available for high-risk areas.

How Cholera Toxin Causes Watery Diarrhoea



1 *V. cholerae* attaches with toxin-coregulated pili

2 cholera toxin B subunits bind GM1 on the cell

3 the A subunit enters and switches adenylate cyclase on

4 chloride is secreted; sodium uptake is blocked

5 water follows: up to 1 litre per hour of rice-water stool

Why oral rehydration solution works

the sodium-glucose co-transporter is not affected by the toxin, so glucose carries sodium - and water - back into the body. ETEC heat-labile toxin (LT) acts by exactly the same mechanism.

Aeromonas hydrophila

- *A. hydrophila* is a common inhabitant
  - of fresh and salt water, especially in warm summer months.
- *A. hydrophila* belongs to the family Aeromonadaceae. It is a Gram-negative, facultatively anaerobic, catalase- and oxidase-positive, glucose-fermenting rod, motile by a single polar flagellum (*A. media* and *A. salmonicida* are non-motile), and mesophilic or psychrotrophic.
- It does not grow at high salt concentrations (above about 5% NaCl).
- It grows optimally at about 28 °C.
- It can produce mildly heat-resistant extracellular lipases and proteases.
- It is common in seawater, well water, sink drains, sewage and a wide range of seafood, including wild and farmed fish, raw and cooked shrimps, oysters, crabs and scallops, and on vegetables.

Aeromonas hydrophila

- Symptoms appear within 24 hours after the ingestion of food containing viable *A. hydrophila* cells.
- The infective dose is not well defined; estimates range from 10^4 to 10^{10} cells.
- The commonest symptom is watery diarrhoea,
  - sometimes with fever and vomiting.
- The disease is generally self-limiting and resolves within 2 weeks.

Prevention and control

- store food below 5 °C, bearing in mind that the organism is psychrotrophic;
  - cook seafood thoroughly and disinfect drinking water;
- organic and inorganic acids are effective against *A. hydrophila*;
- the vegetative cells are easily destroyed by heating at 56 °C for 10 minutes,
- but some of the exotoxins and exoenzymes of *Aeromonas* are heat-stable and may survive this treatment.

MOULD INTOXICATIONS FROM FOOD

MYCOTOXICOSIS

- Mycotoxins are toxic secondary metabolites produced by moulds; they are not antigenic and cause disease in humans and animals. Intoxication caused by eating food or feed contaminated with mycotoxins is called mycotoxicosis.
- At least 14 mycotoxins are carcinogenic, and most cause acute or chronic disease.
- Some mycotoxicoses are of historical importance and caused epidemics in the past, especially in Russia, Japan and Europe: alimentary toxic aleukia caused by *Fusarium* species, ergotism caused by *Claviceps purpurea* and yellow rice disease caused by *Penicillium* species.
- More than 400 mycotoxins are known today; the most important producing genera are *Aspergillus, Penicillium, Fusarium* and *Alternaria*.

MYCOTOXICOSIS

- Since the discovery of aflatoxin in the early 1960s, toxic secondary metabolites of moulds have been studied intensively for more than 60 years.
- To protect people from their effects, maximum permitted levels of mycotoxins in food and feed are laid down by law (in the EU and Türkiye, Regulation (EU) 2023/915 and the Turkish Food Codex Regulation on Contaminants).
- About 350 mould species are known to produce mycotoxins -
- a small proportion of all moulds; most of the thousands of species tested do not produce them.
- About 18 aflatoxin derivatives are known.
- The ochratoxins comprise seven structurally related compounds, of which ochratoxin A (OTA) is the most important.
- More than 200 trichothecenes are known.
- *Alternaria* species produce more than 30 different toxic metabolites.

MYCOTOXICOSIS

MOULD	COLOR	TOXIN PRODUCER	COMMENTS
Penicillium	Green to green-blue	Yes - Ochratoxin, Citrinin, Patulin	Several potential toxins associated with certain species. Most common toxin producer in silage.
Aspergillus	Yellow-green	Yes - Aflatoxin, Ochratoxin	Found in drought, heat-stressed conditions or insect infested fields.
Fusarium	White to pinkish - white	Yes- Zearalenone, Vomitoxin (DON), T-2 Toxin, Fumonisin	Common in cold, wet seasons. Certain strains produce extremely potent toxins.
Mucor	White / Grey	None	Found especially in sealed corn. Grows at low temperatures. Also found in manures and soils.
Rhizopus	Black/white	None	Requires high moisture and an advanced decay mould. Common bread mould.
Cladosporium	White	None	Produces yeast-like symptoms. Grows at low temperatures.

MYCOTOXICOSIS

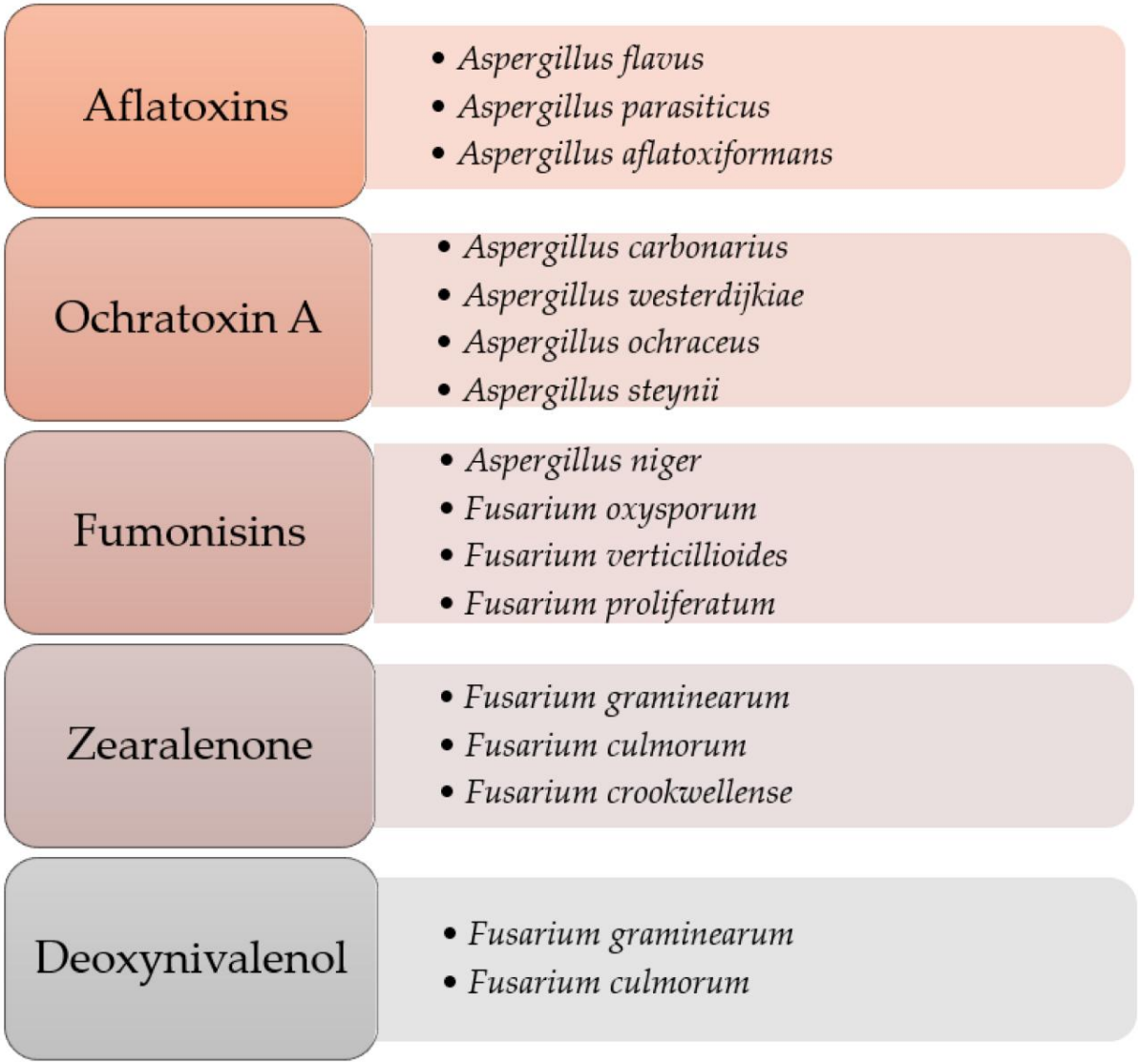
Mycotoxin	Molds	Foods	Toxicities
Aflatoxins	*A. flavus,* *A. parasiticus* *A. nomius*	Peanut, nuts, wheat, corn, rice, pepper, milk, spices, dried fruits, soybean.	Haemorrhage, carcinogenic, digestive tract damage.
Citrinin	*P. citrinum* *P. expansum* *Monascus* spp.	Wheat, barley, rye, oats, corn, rice.	Nephrotoxin (kidney damage), inhibit respiration.
Ochratoxin	*A. ochraceus* *P. verrucosum* *A. carbonarius*	Cereal grains, peanuts, wine, beer, coffee, dried fruits.	Nephrotoxin (kidney damage), kidney tumours, liver damage, enteritis.
Patulin	*P. expansum* *P. griseofulvum*	Feed, apple juice, fruit, vegetable.	Gastrointestinal damage, genotoxic in animals.
Roquefortin	*P. roqueforti*	Blue cheese.	Neurotoxin, liver damage, hemorrhagic in digestive tract.
Zearalenone	*F. culmorum* *F. graminearum*	Corn, cereal crops.	Enlargements of mammary glands, estrogenic effects.

MYCOTOXICOSIS

Mycotoxins	Fungus	Health Effect	Source
Aflatoxins B1,B2,G1,G2, (M1)	Aspergillus and penicillium species	Hepatotoxin carcinogen immunosuppressant	Cereals, maize or corn, tree nuts, ground nuts
Ochratoxins: A, B, C, α, β, γ	Aspergillus and penicillium species	Nephrotoxin, immunosuppressant, renal, and hepatic carcinogens	Vegetables, cereals, vine fruits, wine, beer, coffee
Trichothecenes Toxins: NT-1, NT-2, T-2, HT-2 Diacetoxicirpeaol (DAS) Nivalelol (NIS) Deoxynivalenol (DON) Fusarenon-X	Fusarium species and Gilerellazeac species	Hepatotoxin carcinogen immunosuppressant, neurotoxin, necrosis, malabsorption, skin issues	Grains, rice, oats, cereals, maize or corn
Fumonisins: B1, B2, B3	Fusarium species	Esophagus cancer, liver cancer neurotoxic	Maize or corn, cereals, grains, beer, garlic, beans, asparagus
Zearalenone	Fusarium species	Reproductive issues, infertility, early development	Corn wheat, oats sorghum, sesame seed, hay, silage
Cyclopiazonic acid	Aspergillus and penicillium species	Convulsions	Cheese, maize or corn, ground nuts
Citrinin	Aspergillus and penicillium species	Neurotoxic, nephrotoxic, carcinogen	Rice, cheese, soy, maize or corn, cereals, grains
Patulin	Aspergillus and penicillium species	Neurotoxin, mutagenic, hepatotoxic, immunosuppressant	Fruits, juices, vegetables
Ergot Alkaloids	Claviceps species	Digestive disorders, nervous disorders, seizures, vomiting, headaches	Cereals, grains, maize or corn

MYCOTOXICOSIS

MOULD	MYCOTOXIN
Aspergillus	Aflatoxin
	Ochratoxin
Penicillium	Ochratoxin
Fusarium	DON (Deoxynivalenol)
	ZON (Zearalenone)
	T-2 Toxin
	Fumonisin



MYCOTOXICOSIS

Mycotoxins	Producing microorganisms	Foods mainly affected	Toxic impacts/effects
Aflatoxins	*Aspergillus flavus, Aspergillus parasiticus*	Nuts, groundnuts, pistachios, cereals, millet, also milk to a secondary degree	Hepatotoxic, mutagenic, carcinogenic, teratogenic
Citrinin	*Penicillium citrinum*	Barley, oats, maize, rice, walnuts	Nephrotoxic, mutagenic, carcinogenic (?)
Cyclopiazonic acid	*Aspergillus flavus, Penicillium cyclopium*	Groundnuts, maize, cheese	Neurotoxin
Deoxynivalenol (Vomitoxin)	*Fusarium graminearum, Fusarium culmorum*	Wheat, barley, maize, rye	Neurotoxin, immunotoxic, carcinogenic (?)
Ergot alkaloids	*Claviceps purpurea*	Rye, wheat, oats	Neurotoxin, circulatory disturbances, carcinogenic
Fumonisins	*Fusarium verticolloides*	Maize and products containing maize	Neurotoxin, hepatotoxic, harmful to the lungs, carcinogenic (?)
Moniliformin	*Fusarium verticolloides (moniliforme)*	Maize and products containing maize, wheat (cereals)	Harmful to the heart, hepatotoxic, nephrotoxic
Ochratoxin A	*Aspergillus ochraceus, Penicillium verrucosum*	Cereals, beans, green coffee beans	Carcinogenic, teratogenic, neurotoxic, nephrotoxic
Patulin	*Penicillium patulum, Aspergillus clavatus*	Apples, pears, beans, wheat	Carcinogenic, mutagenic, hepatotoxic
Penicillic acid	*Penicillium puberulum, Aspergillus ochraceus*	Maize, barley, beans	Neurotoxic
Sterigmatocystin	*Aspergillus versicolor, Aspergillus nidulans*	Cereals, green coffee beans, cheese	Teratogenic, skin irritant, carcinogenic (?)
T-2 Toxin/ HT-2-Toxin	*Fusarium sporotrichioides, Fusarium poae*	Maize, barley, oats, millet	Strong neurotoxin, skin irritant, carcinogenic
Zearalenone	*Fusarium graminearum*	Cereals	Mucous membrane irritant, estrogenic, causing infertility, carcinogenic (?)

TOXINS PRODUCED BY *FUSARIUM* SPECIES

- Many *Fusarium* species produce mycotoxins.
  - The largest group is the trichothecenes, with more than 200 derivatives; T-2 toxin, deoxynivalenol (DON, vomitoxin) and a few others occur naturally in cereal products and feed.
  - Other *Fusarium* toxins include zearalenone, moniliformin, fusarin C and the fumonisins. Trichothecenes such as T-2 toxin caused alimentary toxic aleukia (ATA) in the former Soviet Union in the 1940s.

Fumonisins

- Fumonisins occur mainly in maize, but also in other cereals. They were first isolated in 1988 from *Fusarium verticillioides* (formerly *F. moniliforme*). Several types are known; fumonisin B_1 (FB_1) is the most toxic and the most common.

TOXINS PRODUCED BY *PENICILLIUM* SPECIES

Rubratoxin

- Rubratoxin, produced by *Penicillium rubrum*, causes a haemorrhagic disease in cattle, pigs and chickens. It has been isolated from soil and from peanuts, maize, legumes and sunflower seeds.

Patulin

- Patulin was first isolated as an “antibiotic”, and its antibacterial and antifungal effects have been reported. It is genotoxic and immunotoxic in animals; IARC places it in Group 3 (not classifiable as to carcinogenicity in humans).
- *Penicillium expansum*, the blue mould of apples, is the main producer in fruit. Apples and apple products (juice, purée) are the main sources; pears, peaches and other fruits can also be affected. The EU limit is 50 µg/kg in fruit juices (10 µg/kg in foods for infants).
- Patulin is heat-stable and survives boiling at 100 °C for 15 minutes, so the pasteurisation of fruit juices does not destroy it. Alcoholic fermentation, however, largely destroys it, and removing rotten fruit before pressing is the most effective control.

TOXINS PRODUCED BY *ASPERGILLUS* SPECIES

Ochratoxins

- Ochratoxins are produced by species of the *Aspergillus ochraceus* group, by *A. carbonarius* (in grapes) and by *Penicillium verrucosum* (in cereals in cooler climates); ochratoxin A (OTA) is the most toxic.
- OTA is found in cereals, legumes, peanuts, red and black pepper, coffee, cocoa, dried vine fruits, wine, beer, some fermented fish products and animal feed, and in animal tissues such as pig kidney and blood.

Aflatoxins

- Aflatoxins were discovered after “Turkey X disease” killed more than 100 000 turkeys in England in 1960. The cause was traced to *Aspergillus flavus* growing in Brazilian peanut meal added to the feed, and the toxin was named aflatoxin (*A. flavus* toxin).
- *A. flavus* and *A. parasiticus* are the main producers; *A. nomius* and a few related species also produce aflatoxins.
- Aflatoxins can be found in any food on which these moulds grow.
- They are most often found in peanuts, maize and other cereals, tree nuts (pistachios, hazelnuts), dried figs, red pepper and spices. Aflatoxin M_1 appears in the milk of animals fed aflatoxin-contaminated feed. For Türkiye's pistachio, hazelnut and dried fig exports, aflatoxin control is of major economic importance.

Aflatoxin: From Field to Liver

1	Field	*A. flavus* infects nuts and maize - favoured by drought stress and insect damage
2	Drying	slow drying or rain at harvest: mould grows above aw 0.80 at 25 - 35 °C
3	Storage	warm, humid stores: aflatoxin B_1 accumulates - pistachios, hazelnuts, dried figs, red pepper
4	Feed to milk	dairy cows turn 1 - 3% of dietary B_1 into aflatoxin M_1 in their milk
5	Liver	B_1 is activated to an epoxide that binds DNA - liver cancer, risk multiplied by hepatitis B

EU maximum levels (Reg. 2023/915)

µg/kg, ready-to-eat: B_1 / total

groundnuts 2 / 4

hazelnuts 5 / 10 pistachios 8 / 10

dried figs 6 / 10 milk M_1 0.050

Control

rapid drying, clean dry storage

sorting: contaminated figs glow bright greenish-yellow under UV light

sampling and testing of every lot