# Characteristics of major food pathogens


FOOD QUALITY AND SAFETY - Prof. Angel Angelov, Assoc. Prof. Velitchka Gotcheva

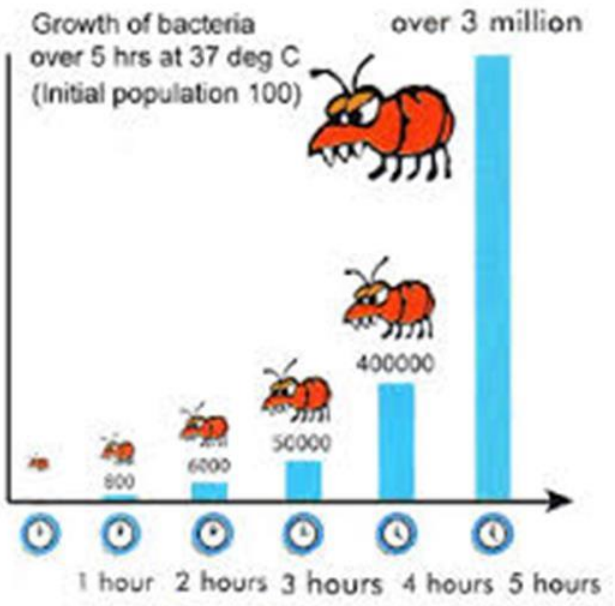
angelov@uft-bio.com, gotcheva_v@uft-bio.com

# Microorganisms in foods

- Food spoilage
- Food-borne diseases and food poisoning

Exponential growth of microorganisms

| Time | cfu/g (ml) |
|---|---|
| 0 h | 1 000 |
| 0.5 h | 2 000 |
| 1 h | 4 000 |
| 1.5 h | 8 000 |
| 2 h | 16 000 |
| 5 h | 1 000 000 |

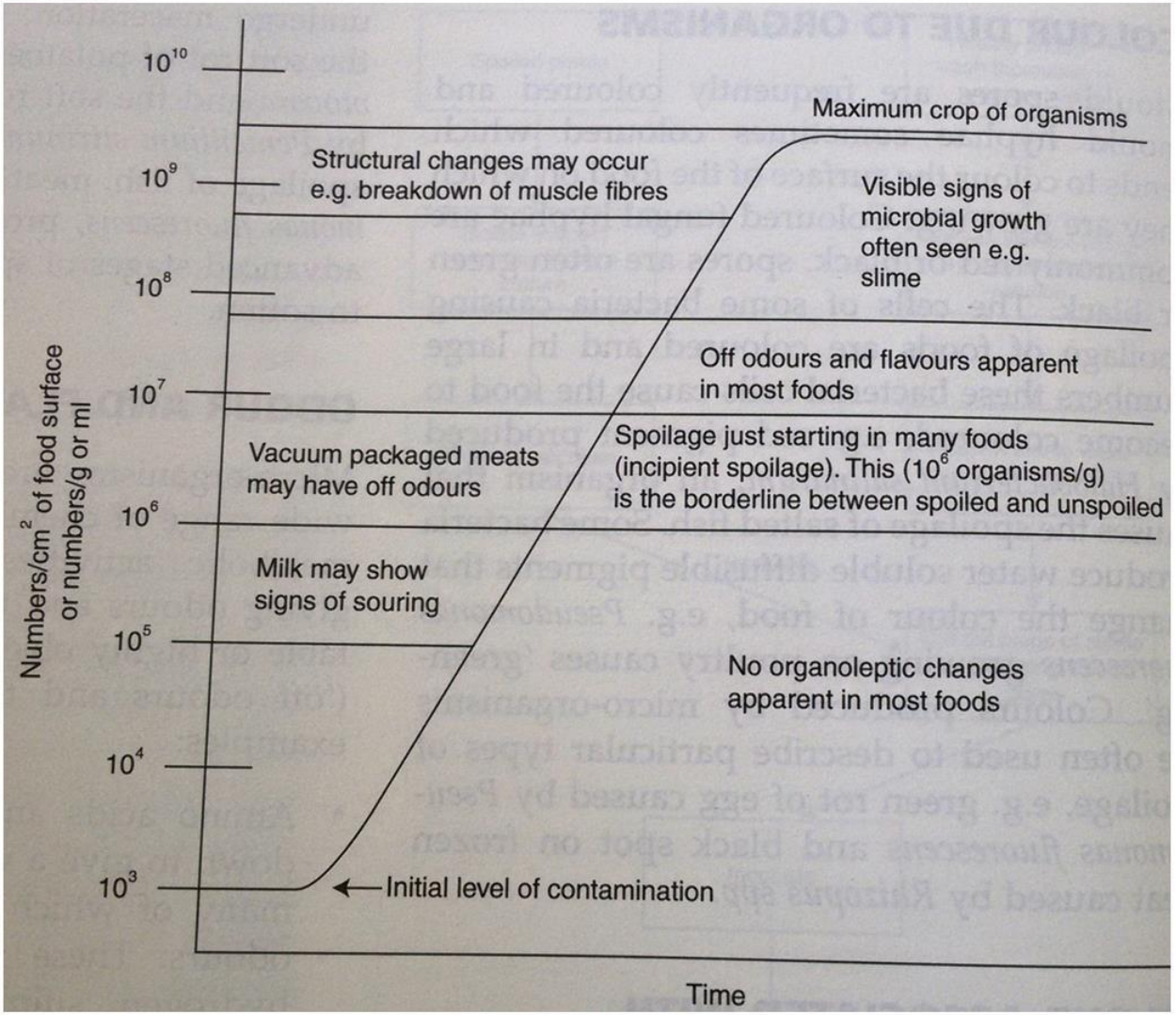


# Food spoilage

- General appearance
- Colour
- Odour and flavour
- Texture
- A mixture of above signs

Low risk because of organoleptic signs of microorganisms present.

# Food spoilage



# Food-borne diseases

- Disease – any harmful change in tissue and/or metabolism that produces the symptoms of illness
- Natural resident microflora (microbiome)
- Transient microflora
- **Pathogens** – microorganisms which cause diseases by invading tissues and/or producing toxins

# Diseases caused by bacteria

**Production of exotoxins**

- Proteins synthesized by metabolic activity
- Produced by **Gr+ and Gr-** organisms
- Non-structural components of bacterial cell
- Secreted from the cell into the environment

# Diseases caused by bacteria

**Production of endotoxins**

- Lipopolysaccharides
- Toxic components of the cell wall that are released upon cell lysis
- Produced by **Gr-** microorganisms

# Diseases caused by bacteria

**Effect of endotoxins**

- Enterotoxins in the gut → diarrhea and abdominal pain
- Stimulate release of pyrogens → fever
- Escape of blood from skin capillaries → rashes
- Increase in the permeability of blood capillaries → lowered blood pressure, accumulation of blood in various organs → waste metabolic products are not removed and organs starve for oxygen and nutrients → septic shock
- Prolonged effects can lead to irreversible tissue and organ damage and death!

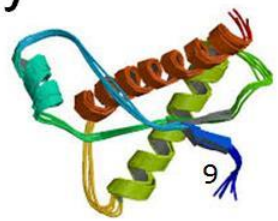
# Diseases caused by viruses and prions

## Viruses

- Invade host cells, take over host cell metabolism and induce the cell to produce new virus particles → destruction of host cells

## Prions

- Protein character, significant thermal stability, resistance to cellular proteases
- Disease - BSE "bovine spongiform encephalopathy" Creutzfeldt-Jakob or “mad cow” disease



# Food infections

- Ingestion of foods containing **live** pathogenic MO (bacteria, viruses, parasites) above a certain number (infective dose ID)
- MO grow in the gastro-intestinal tract → inflammation
- Infections - certain bacteria, viruses and all parasites
- Symptoms: after 6-24 h or more - fever, headache, dizziness, vomiting, diarrhea, abdominal pain

# Food infections

- **Bacteria**: *Salmonella spp*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Yersinia enterocolitica*
- **Viruses**: hepatitis A, Norovirus, Rotavirus
- **Parasites**: *Trichinella spiralis, Anisakis simplex, Giardia duodenalis, Toxoplasma gondii, Cryptosporidium parvum, Cyclospora cayetanensis*
- **Infective dose** (ID) - the number of pathogenic cells that causes infection of the host
- ID - specific to each type MO, depends on the overall health and the age of host

# Food intoxications

- Consumption of food containing **toxins** produced by MO
- Presence of live MO in food is irrelevantat
- **Exotoxins** – enterotoxins (*Staphylococcus aureus*), Shiga-toxins (*Shigella*, some *E. coli*), neurotoxins (*Clostridium botulinum*)
- **Mycotoxins** – produced by moulds or algae

# Food-borne diseases - mechanisms

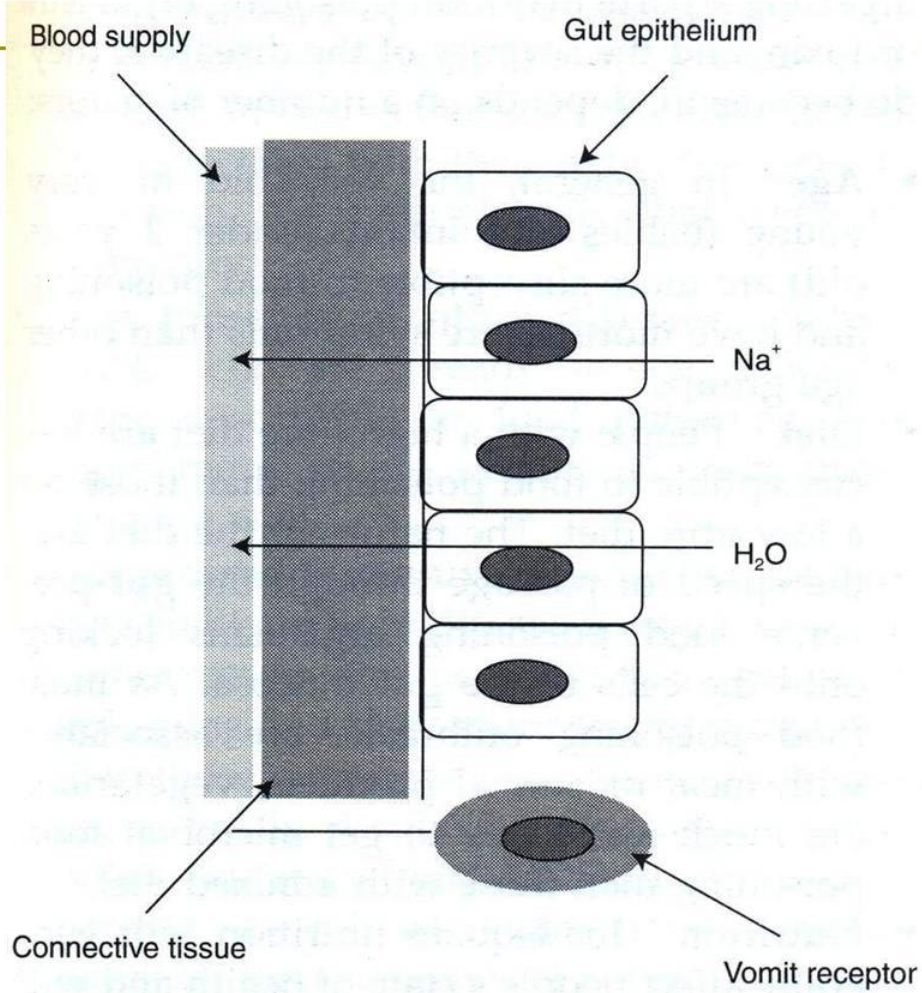



**Figure 9.6** How normal gut physiology operates in relation to sodium ions and water

# Food-borne diseases - infections

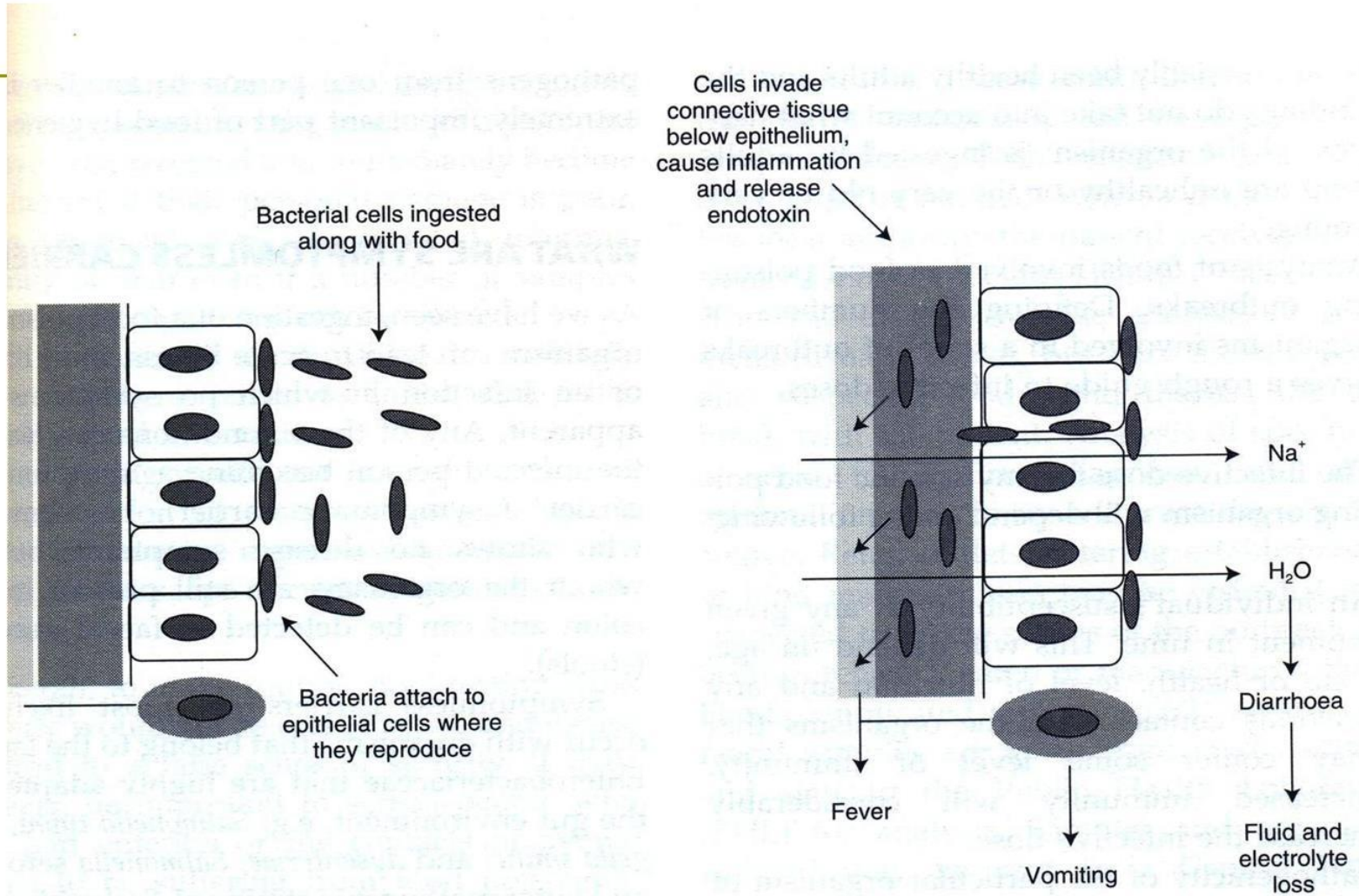



**Figure 9.9** What happens when infective *Salmonella* cells are ingested

# Food-borne diseases - enterotoxins

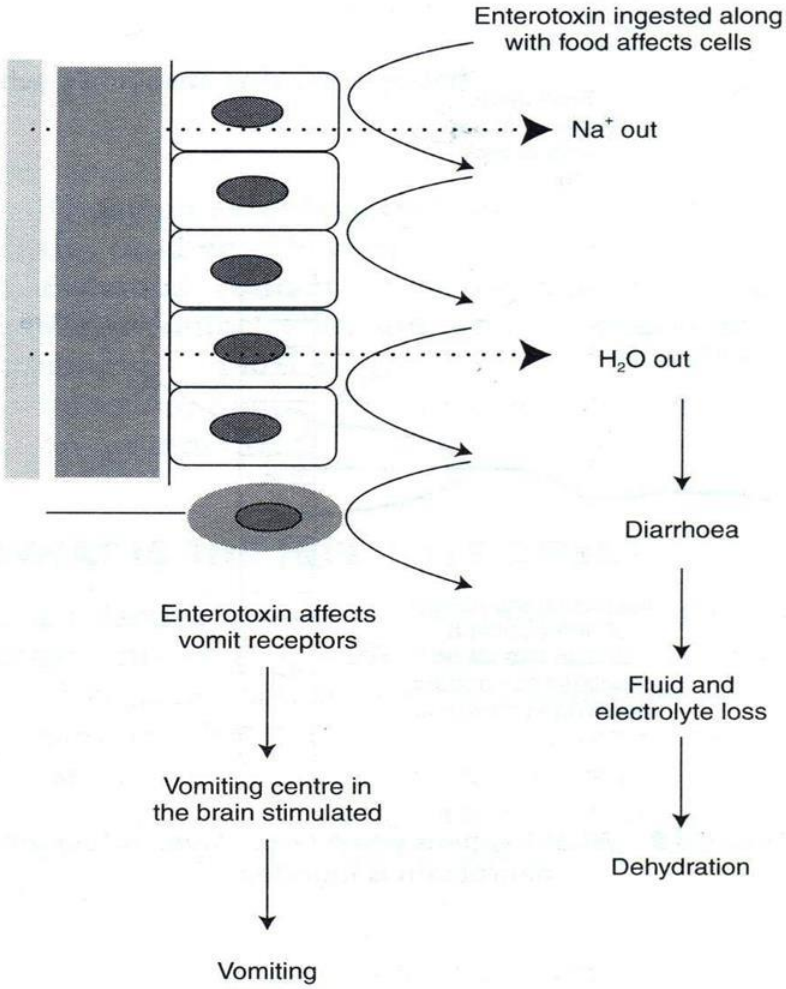



**Figure 9.7** What happens when an enterotoxin is ingested with food

# Food-borne diseases – neurotoxins

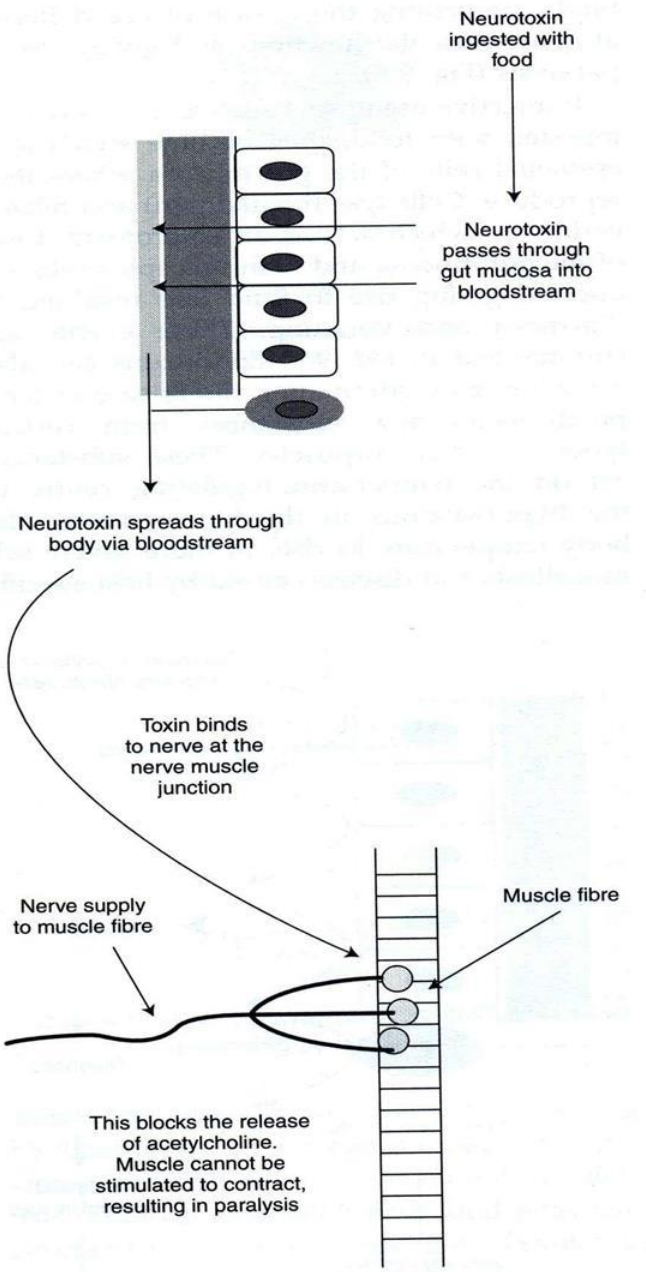



**Figure 9.8** What happens when *Clostridium botulinum* neurotoxin is ingested

# Enterobacteriaceae

- *Salmonella*
- *Escherichia coli*
- *Yersinia pestis*
- *Klebsiella*
- *Shigella*
- *Proteus*
- *Enterobacter*
- *Serratia*
- *Citrobacter*

PATHOGENS

**Common characteristics**

- $Gr^-$ rods,
- facultative anaerobes,
- oxidase- (do not produce cytochrome C oxidase)

# Salmonella

- Gr- rods, not spore-forming, facultative anaerobes, most produce hydrogen sulfide (HS) – detection in media with ferrous sulphate, over 2500 serovars
- Two species – *S. enterica* (warm-blooded animals, environment) and *S. bongori* (cold-blooded animals, esp. reptiles)
- One of the most common causes of food poisoning

# Salmonella

- **Distribution**: intestinal tract of wild and domestic animals; flies; reptiles (snakes, turtles, lizards), amphibians (frogs), birds (baby chicks), excreted in faeces and remains alive for long periods of time (years), sewage, soil, surface water, feed, pet foods

- **Food:** Contaminated eggs, poultry, meat, unpasteurized milk or juice, cheese, contaminated raw fruits and vegetables (alfalfa sprouts, melons), spices, and nuts

# Salmonella


COUNTERTHINK - "WHEEL OF SALMONELLA"
WHEEL OF SALMONELLA
KALE
TOMATOES
SPINACH
PEPPERS
LETTUCE
CILANTRO
PARSLEY
ONIONS
ALFALFA

# Salmonella

**Salmonelosis**

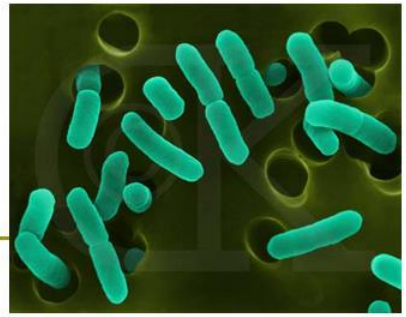
- Infectious dose: $10^6$-$10^9$ cells
- Incubation Period: 12-72 h
- Symptoms: diarrhea, fever, abdominal cramps, vomiting
- Duration: 4-7 days and most people get better without treatment
- More serious illness in older adults, infants, and persons with chronic diseases

# Salmonella

**Control**

- Highly resistant - 5-46ºC, pH 3.8-9.5, not destroyed by freezing
- UV light and heat accelerate destruction - heating to 55°C for 90 min, or to 60°C for 12 min.
- Heat food for at least 10 minutes to an internal temperature of 75°C
- Adequate hygienic practices in animal husbandry and poultry production of meat, eggs and milk
- Storage of raw materials and foods at T up to 5°C

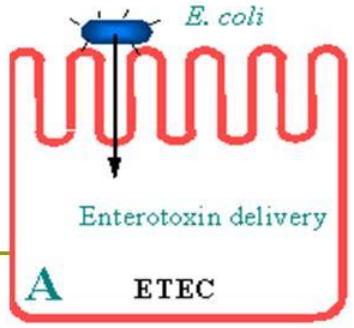
# Escherichia coli



- 7 groups, commonly found in the lower intestine of warm-blooded animals
- Most strains are harmless, part of normal gut flora, even beneficial for the host
- Enterotoxigenic *E. coli* (ETEC)
- Enteropathogenic *E. coli* (EPEC)
- Enteroinvasive *E. coli* (EIEC)
- Enterohaemorrhagic *E. coli* (EHEC)
- Enteroaggregative *E. coli* (EAEC)

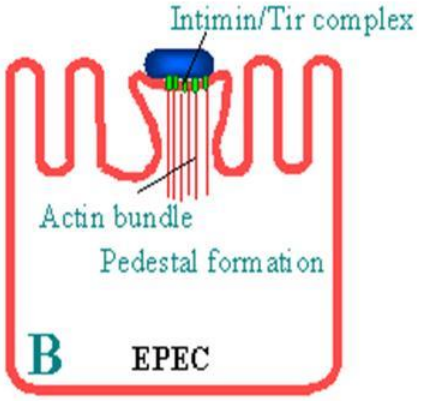
PATHOGENS

# Enterotoxigenic E. coli (ETEC)



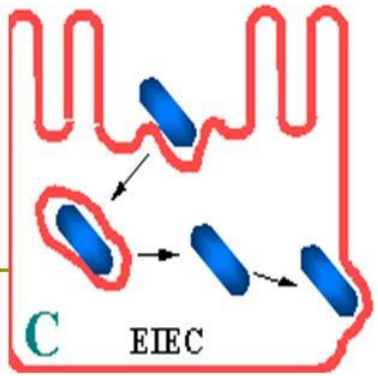

- Main cause of diarrhea in developing countries
- The most frequent cause of diarrhea associated with travel
- 210 M cases, 380,000 deaths a year, mainly in children
- Cells attach with fimbria to intestinal cells of host, expression of enterotoxins, does not destroy the cell wall

# Enteropathogenic E. coli EPEC




- The most widespread among children, especially in developing countries (less than 5 years-old)
- Fecal-oral transmission, incubation period: 8 h to 12 days
- Destroys microvilli of intestinal cells and attaches to them, expression of enterotoxins

# Enteroinvasive *E. coli* EIEC




- Symptoms similar to shigellosis
- Incubation period: 12-72 hours, blood and mucus in the stool
- Highly invasive, penetrate the intestinal cells and multiply therein - heavy damage of gut wall, gut cell destruction

# Enterohaemorrhagic *E. coli* (EHEC)


- Disease mechanism - like EPEC but produce Shiga toxin → destruction of ribosomes → death of intestinal cells
- Children and adults - hemolytic uremic syndrome (HUS) - destruction of red blood cells → blockage of the kidney (2-7% of cases)
- **Distribution**: Digestive system of cattle (corn) - contaminated ground meat, raw milk, swimming / drinking contaminated water, contaminated fruits / vegetables

# Enterohaemorrhagic *E. coli* (EHEC)


- Most dangerous: *E. coli* O157:H7, O121, O26, O103, O111, O145, O104:H21

***E. coli* O157:H7**

- Infection via the faecal-oral route, raw milk from goats sheep and cattle, contaminated raw leaf green vegetables, undercooked meat and raw milk
- Infectious dose: 10 – 100 cells, incubation period 3-4 days

# Enteroaggregative *E. coli* (EAEC)


- "Stacked brick" pattern of adhesion to the human epithelial cell
- Aggregates and colonizes the intestinal mucosa, releasing enterotoxins and cytotoxins that damage host cells and induce inflammation → diarrhea and other gastrointestinal symptoms
- Emerging enteric pathogen – first reported in 1987, common bacterial cause of paediatric diarrhea, especially in developing countries, associated with traveller's diarrhoea and infections in HIV-infected individuals, cause of sporadic food-poisoning outbreaks in the developed world

# Shiga-toxin *E. coli* (STEC)

***E. coli* O104:H4**

- Combines virulence factors of EHEC and EAEC
- Outbreak in Europe in June 2011 - 4,075 cases (incl. 908 cases complicated by HUS) and 50 deaths in 16 countries


EFSA Journal 2011;9(10):2390

**SCIENTIFIC REPORT OF EFSA**

**Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: Taking Stock[1]**

**European Food Safety Authority[2, 3]**

European Food Safety Authority (EFSA), Parma, Italy

# Shigella

- Causes diarrhea to humans and primates, most commonly seen in child-care settings and schools, cause of traveler's diarrhea from contaminated food and water in developing countries

- Sources: Contaminated food or water, contact with an infected person. Foods most often associated with *Shigella* outbreaks are salads and sandwiches that involve a lot of hand contact in their preparation, and raw vegetables contaminated in the field.

# Shigella

- Infectious dose: ~100 cells, incubation period 1-7 (1-3) days
- Damages gut epithelium, some strains produce enterotoxin and shiga-toxin
- Symptoms: sudden abdominal cramping, fever, diarrhea that may be bloody or contains mucus, nausea and vomiting
- Highest risk for children 2-4 years old

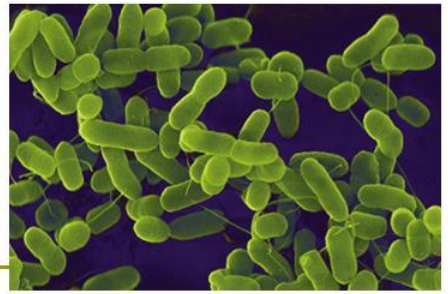
# Campylobacter

- Gr-, spirals, microaerophilic, motile, with one- or tw[illegible] flaggela, oxidase-positive
- Genetically related to *Helicobacter* sp.
- 16 species, *C. jejuni* and *C. coli* - most common pathogens
- Most strains produce a toxin that inhibits cell division and activation of the immune system
- ID: 100-500 cells, intestinal inflammation, diarrhea, colics, fever, 5-7 days
- Incubation period: 1-10 days (3-5 days)

# Campylobacter jejuni

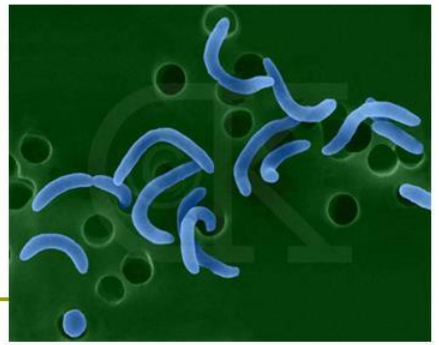
- **Distribution**: gastrointestinal tract of wild and domestic animals, surface water, infected carcass of animals and birds through fecal contamination during processing
- **Risk foods**: raw milk (from contamination or mastitis), dairy products, water, meat products, poultry (USA: 90-100% of poultry meat is contaminated, cause of 70% of campylobacteriosis)
- Rarely found in thermally processed foods

# Yersinia enterocolitica



- Gr- cocci, zoonotic disease (cattle, deer, pigs, poultry)
- **Symptoms**: acute diarrhea, chronic lymphadenopathy, liver inflammation, enterocolitis, fever , etc.
- “Loves iron" - often contaminates stored blood
- **Incubation period**: 24-36 h, lasts 5-14 days to months, ID unknown
- **Sources**: GIT of animals, seafood! - Scandinavia, northern regions of the US
- Psychrotrophic, heat-sensitive

# Vibrio sp

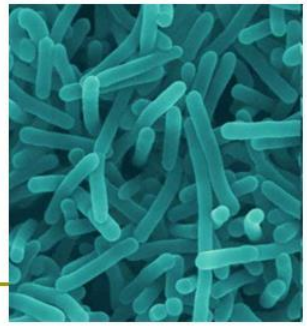


- Gr- rods, curved, motile, polar flaggela, facultative anaerobes, oxidase-positive
- **Distribution**: salt water, low nutritional requirements
- **Infections**: poorly cooked seafood – gastroenteritis; in open wounds - septicemia
- *V. cholerae* - from contaminated water, feces → water (sweet, salt water) → food
- Cholera toxin (holeragen) - rapid dehydration

# Vibrio sp

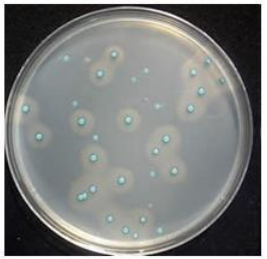
- *Vibrio vulnificus* - warm climate, small-scale but lethal epidemics (New Orleans after Katrina, almost every year - Florida)
- *V. parahaemolyticus* - saltwater seafood
- Incubation period: 24 h, diarrhea, nausea, vomiting, colics, fever, 72h to 10 days

# Listeria monocytogenes



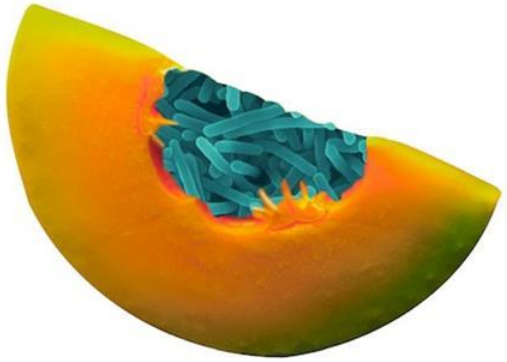
- Gr+ rods, facultative anaerobes, motile at ~ 30°C
- One of the most virulent food-borne pathogens - 20-30% of disease leading to death
- The third most common cause of meningitis in newborns
- ID: unknown; incubation period: 30-90 days
- Listeriosis - septicemia, meningitis, encephalitis, pneumonia, uterine infections - miscarriages and other
- Symptoms: fever, nausea, vomiting, diarrhea - often before the more serious manifestations of the disease

# Listeria monocytogenes



Oxoid Chromogenic Listeria Agar ( OCLA )

- Resistance to NaCl, nitrites, low pH; growth at 3°C
- **Risk foods**: raw or improperly pasteurized dairy products (soft cheese), ice cream, fresh vegetables, sausages, raw meat products, raw and smoked fish
- **Control**: prevent contamination, food storage at 0°C, adequate heat treatment



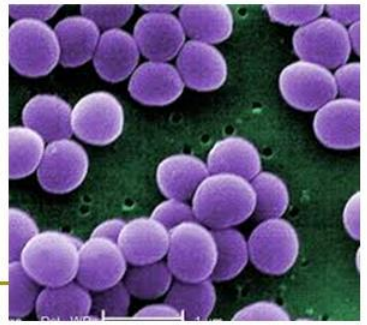
# Clostridium botulinum

- Gr+ rods, obligate anaerobes, form endospores
- Botulinum neurotoxin A-G, secreted in food → soft muscle paralysis; incubation time: 12-36 h, can cause death
- Distribution: soil → surface water, marine sediment, vegetables, fruits, honey, water, raw meats
- Inappropriate canning regime (time / temperature)
- *C. botulinum* can transfer neurotoxin genes to other clostridia!

# Clostridium botulinum

- **Risk**: Low-acid foods (pH> 4.6), $a_w$> 0.93, anaerobic-packaged raw foods, insufficient heat treatment, low content of preservatives (salt, nitrite)
- **Control**: killing of spores - 121°C for 3 min ($10^{12}$ cells of the most heat-resistant strain); conditions preventing spores development; refrigerated storage of raw foods

# Staphylococcus aureus



- Gr+ cocci, facultative anaerobes, grapes-shaped clusters, catalase positive, "golden staph"
- Skin microflora - skin (20% of people), nose and throat (30-50% of people)
- Staphyloxanthin - a carotenoid pigment, virulent factor with antioxidant effect → prevents the action of the immune system against the pathogen
- Some strains - enterotoxin → gastroenteritis (nausea, vomiting, diarrhea, colic)
- Number of cells to accumulate toxin - $5x10^6$ cfu/g, risk - 1 ng/g toxin; incubation period - 0.5-6 hours

# Staphylococcus aureus

- Skin infections, pneumonia, meningitis, osteomyelitis, endocarditis, chest pain, sepsis
- **Sources**: skin, soft tissue, breathing, blood vessels, infected wounds → towels, bedding, clothing
- **Risk foods**: cured meat products, milk-containing foods, fish, seafood, canned vegetables, pasta
- Survives for hours, days, weeks, even months on dry surfaces

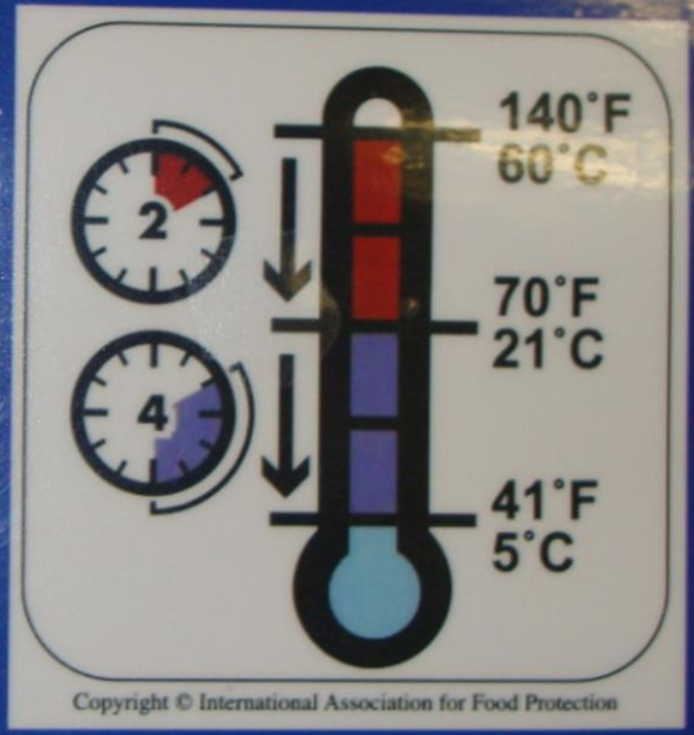
# Staphylococcus aureus

**Control**

- Use disposable gloves in manual handling of food
- Refrigerated storage of raw materials
- After cooking, quick chilling of risk food to temperature below 5°C
- Prevent contact of workers with cough, runny nose, cuts and burns with food and contact surfaces

# Cooling Methods

- Hot Foods
  - 60°C (140°F) to 21°C (70°F) [illegible] hours then [illegible] (41°F) in 4 hrs
- Ambient Foods
  - Cool to 5°C (41°F) in 4 hours
- Logs





VESSEL SANITATION PROGRAM

| Microorganism | Growth temperature | pH | Water activity ($a_w$), min |
|---|---|---|---|
| *Bacillus cereus* | 4 – 55°C | 5.0 – 8.8 | 0.93 |
| *Campylobacter jejuni* | 32 – 45°C | 4.9 – 9.0 | 0.98 |
| *Clostridium botulinum* | 10 - 48°C | 4.6 | 0.94 |
| *Clostridium perfingens* | 12 – 50°C | 5.5 – 9.0 | 0.97 |
| *Escherichia coli O157:H7* | 7 – 46°C | 4.4 – 9.0 | 0.95 |
| *Listeria monocytogenes* | -0.4 – 45°C | 4.4 – 9.4 | 0.92 |
| *Salmonella spp.* | 5 – 46°C | 3.8 – 9.5 | 0.94 |
| *Staphylococcus aureus* | 7 – 48°C | 6.0 – 7.0 | 0.83 |
| *Yersinia enterocolitica* | -1.3 - 42°C | 4.2 – 9.6 | - |

| Organism | Temperature Minimum °C/°F | Temperature Optimum °C/°F | Temperature Maximum °C/°F |
|---|---|---|---|
| *Aeromonas hydrophlia* | >1->4/<34->39 | 28-35/82-95 | >42-45/<108->113 |
| *Aspergillus flavus* (aflatoxin production) | 10/50 | 33/91 | 43/109 |
| *Bacillus cereus* | 4/39 | 30/86 | 50-55/122-131[a] |
| *Brucella* | 6/43 | 37/99 | 42/108 |
| *Campylobacter jejuni* | 32/90 | 42/108 | 45/113 |
| *Clostridium botulinum* type A (toxin production) | 4/39 | 37/99 | 50/122 |
| *Clostridium botulinum* type B (toxin production) | 3/37 | 37/99 | 50/122 |
| *Clostridium botulinum* type E (toxin production) | 4/39 | 29/84 | 45/113 |
| *Clostridium botulinum* type F (toxin production) | 4/39 | 29/84 | 45/113 |
| *Clostridium perfringens* | 12/53 | 46/115 | 50/122 |
| *Escherichia coli* (pathogenic) | 7/45 | 37/99 | 46/115 |
| *Listeria monocytogenes* | -0.4/31 | 37/99 | 45/113 |
| *Plesiomonas shigelloides* | 8/46 | 30/86 | 45/113 |
| *Salmonella* | 5.2/41[a] | 35-43/95-109 | 46/115 |
| *Shigella* | 5/41 | 37/99 | 47/117 |
| *Staphylococcus aureus* | 7/45[b] | 37/99 | 48/118[b] |
| *Streptococcus pyogenes* | >10/>50 | 37/99 | <45/<113 |
| *Vibrio cholerae* | 10/50 | 37/99 | 43/109 |
| *Vibrio parahaemolyticus* | 5/41[a] | 37/99 | 43/109 |
| *Vibrio vulnificus* | 8/46 | 37/99 | 43/109 |
| *Yersinia enterocolitica* | -1.3/30 | 25-37/77-99 | 42/108 |

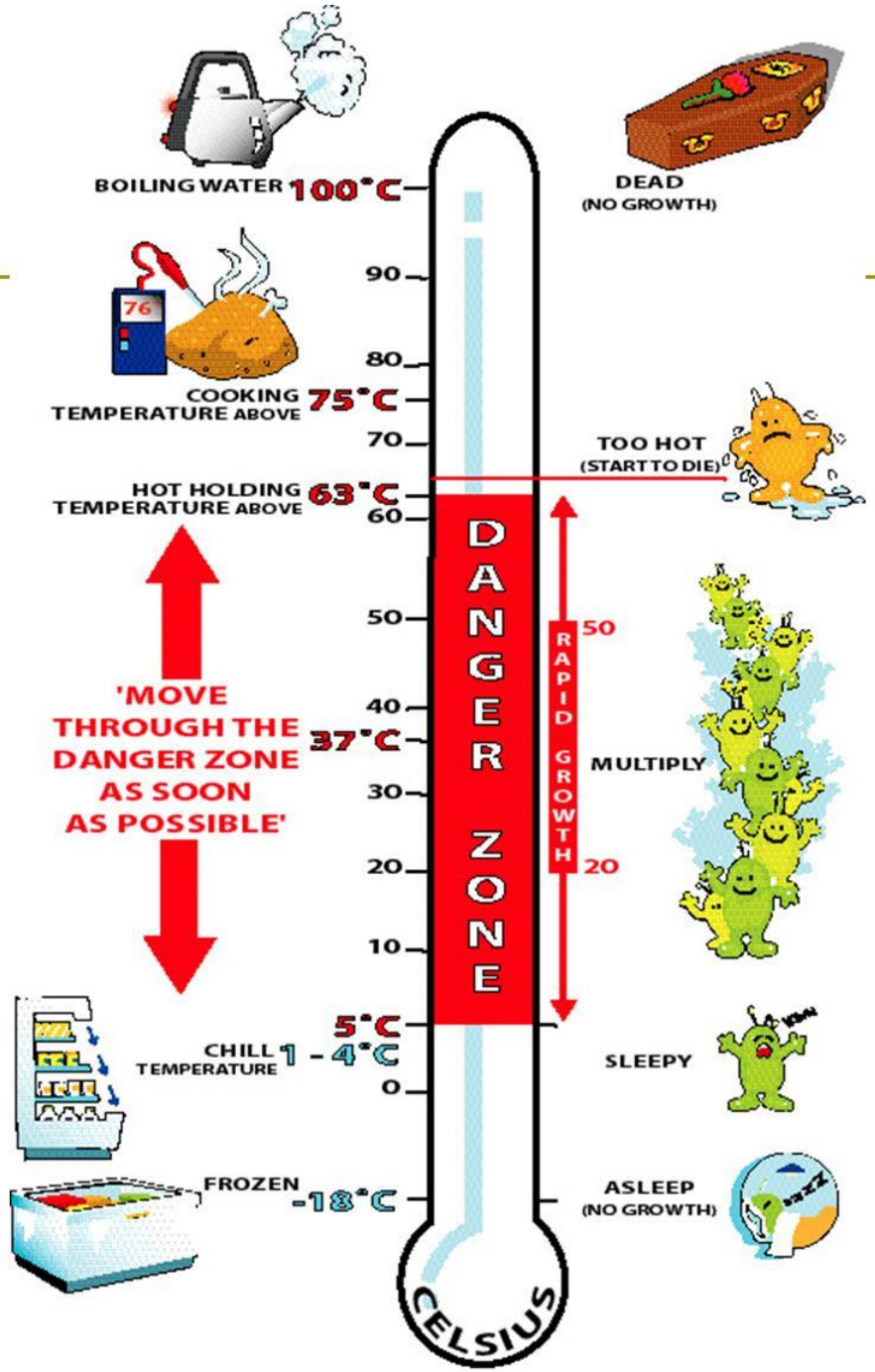
[a] Values for some strains vary and may differ slightly from the listed data.
[b] Minimal temperature for enterotoxin production is 14C (57.2F); maximal temperature for enterotoxin production is 45C (113F).

*Table 1. Optimal and limiting temperatures (rounded to nearest whole number) influencing growth of common foodborn pathogens with other conditions optimum or near optimum (e.g., pH and water activity).*

| Organisms | pH[a] Minimum | pH[a] Optimum | pH[a] Maximum | Water activity[b] Minimum | Water activity[b] Optimum |
|---|---|---|---|---|---|
| *Aeromonas hydrophlia* | <4.5 | 7.2 | - | - | - |
| *Aspergillus flavus* (aflatoxin production) | 2 | 5-8 | >11 | 0.82 | 0.98 |
| *Bacillus cereus* | 5 | 6-7 | 8.8 | 0.93 | - |
| *Brucella* | 4.5-5.1 | 7.3-7.5 | 8.2-8.8 | - | - |
| *Campylobacter jejuni* | 4.9 | 6.5-7.5 | ~9 | >0.987 | 0.997 |
| *Clostridium botulinum* type A (toxin production) | 4.8 | ~7 | 8.5 | 0.95 | - |
| *Clostridium botulinum* type B (toxin production) | 4.8 | ~7 | 8.5 | 0.95 | - |
| *Clostridium botulinum* type E (toxin production) | 5 | ~7 | 8.5 | 0.97 | - |
| *Clostridium botulinum* type F (toxin production) | - | ~7 | - | - | - |
| *Clostridium perfringens* | 5.5-5.8 | 7.2 | 8-9 | 0.96 | ~0.99 |
| *Escherichia coli* (pathogenic) | 4.4 | 6-7 | 9 | 0.95 | 0.995 |
| *Listeria monocytogenes* | 4.4 | 7 | 9.4 | 0.92 | - |
| *Plesiomonas shigelloides* | 4 | 7 | 9 | - | - |
| *Salmonella* | 3.8 | 7-7.5 | 9.5 | 0.945 | 0.99 |
| *Shigella* | 4.9-5 | ~7 | 9.3 | - | - |
| *Staphylococcus aureus* | 4 | 6-7 | 10 | 0.83 | 0.98 |
| *Streptococcus pyogenes* | *4.8-5.3* | *7* | *<9.3* | - | - |
| *Vibrio cholerae* | 5 | 7.6 | 9.6-11 | 0.97 | 0.984 |
| *Vibrio parahaemolyticus* | 4.8 | 7.8-8.6 | 11 | 0.94 | 0.981 |
| *Vibrio vulnificus* | 5 | 7.8 | 10 | 0.96 | 0.98 |
| *Yersinia enterocolitica* | 4.2 | 7.2 | 9.6 | - | - |

[a] Values for some strains vary and may differ slightly from the listed data.
[b] Data not available for optimum water activity, but it is usually 0.99 (with the exception of V. parahaemolyticus that is halophilic and below this value.

*Table 2. Optimal and limiting pH and water activity values influencing growth of common foodborne pathogens.*


BOILING WATER 100°C
DEAD
(NO GROWTH)
90
80
COOKING
TEMPERATURE ABOVE 75°C
70
TOO HOT
(START TO DIE)
HOT HOLDING
TEMPERATURE ABOVE 63°C
60
DANGER ZONE
50
RAPID GROWTH
50
'MOVE
THROUGH THE
DANGER ZONE
AS SOON
AS POSSIBLE'
40
37°C
MULTIPLY
30
20
20
10
5°C
CHILL
TEMPERATURE 1 - 4°C
SLEEPY
0
FROZEN -18°C
ASLEEP
(NO GROWTH)
CELSIUS

# General control measures

- Prevention of primary contamination
- Temperature control (storage)
- Heat treatment - temperature / time
- Good hygiene practices - working environment, pest control, staff
- Prevention of cross-contamination