Characteristic of genus *Salmonella* and pathogenic strains of *Escherichia coli*

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History

• The first was described and isolated strain *S.typhi* as *typhi bacilus*.

• Name *Salmonella* was proposed and codified according D.E: Salmon, bacteriolog, who identified *S.choleraesuis* in 1885.

• Up to 1914 only **12 serotypes** were described.

• Since 1930 the number of discovered salmonells is quickly growing.

• Designation *Salmonella* was used first time for causative agent of pig illness in **1930** and in 1933 was codified by world salmonella committe.
General characteristics

- Straight rods 0.7 – 1.5 x 2 – 5µm **Gram negative**
- **Motile** by peritrichous flagella, facultatively anaerobic
- Optimal temperature 37 C, **oxidase negative, catalase positive**
- Indol and Voges-Proskauer negative, production of acids and H₂S
- Division into serovars: **Kaufmann-White scheme** – numbers and letters given to different O (somatic), Vi (capsular) and H (flagellar) **antigens**.
- Phage typing - epidemiologic purposes
- 01 bacteriophag of Felix and Callow is highly specific for salmonellae – lysis of more than 98% of strains
Taxonomy

• Today more than 2300 serotypes of salmonellae were described.

• Antigen classification of salmonellae is based on somatic O antigens and flagellar H antigens.

• Somatic O-antigens lipopolysaccharides character. Great variety of these antigens is due to differences in structure of specific saccharide chain, where are many types of glycosidic bonds, anomeric configuration and different acetylation.

• Flagellar H-antigen is protein and is thermolabile.

• H-antigens are two types – antigen of the first and second phase has different primary protein structure.
Taxonomy

• According to study of Le Minor et al. (1982) genus *Salmonella* has only one species and seven subspecies, differing by DNA-DNA hybridization, biochemical, serological characteristics.

• Each of seven subspecies is divided to serovars according the composition of somatic and flagellar antigens.

• Family – *Enterobacteriaceae*
• genus – *Salmonella*
• species – biochemic variants
• serovar = serotype – serologic variants
• phagotype – lysotype
• biochemotype
• Presence of certain genetic plasmids
## Taxonomy

<table>
<thead>
<tr>
<th>genus</th>
<th>Species</th>
<th>subspecies</th>
<th>serovar</th>
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</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td><em>enterica</em></td>
<td><em>enterica 59%</em></td>
<td>Typhimurium</td>
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<tr>
<td></td>
<td></td>
<td>salamae 19%</td>
<td>Enteriridis</td>
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<td></td>
<td></td>
<td>arizonae 4%</td>
<td>Typhi</td>
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<td></td>
<td></td>
<td>diarizonae 13%</td>
<td>Virchow</td>
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<tr>
<td></td>
<td></td>
<td>houtaneae 3%</td>
<td>Heidelberg</td>
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<td></td>
<td></td>
<td>indica 1%</td>
<td>etc.</td>
</tr>
</tbody>
</table>
Infection dose

• Infection dose – considerably varies and depends on many factors including the virulence of the strain and resistance of attacked individuals (risk groups are children and weakened individuals).

• Former data about high infections doses – $10^5$-$10^9$, are today requalified. Data about salmonella infection after digestion of $10^1$-$10^5$ CFU are common.

• Frequent capture of salmonellae in raw foods caused that according Reg. (EU) No.2073/2005 amended No.1441/2007 the zero tolerance of salmonella in ready to eat food.
Salmonella - growth conditions

Atmosphere

• **Facultatively anaerobic**, growth in presence of 20-50% CO₂ on minced beef and boiled crabs.
• Higher tolerance to NaCl under anaerobic conditions.
• **pH tolerance** 4.0 - 9.5
• 3.99 tomatoes
• 9.5 eggs rinse water
• After long exposition to acids - **ATR** Acid Tolerance Response
Salmonella - growth conditions

- $a_w$
- 0.93 instant soup mix
- Resistant to drought
- Growth inhibited by 3 - 4% NaCl
  Salt is more effective at lower temperatures
Salmonella - growth conditions

• Temperature demands
• 2 - 46 °C
• Survives at 2 °C (1 - 2 days) beef and chicken meat
• 4 °C (<10 days) on the egg shells
• 46 °C in cremes, custards on chicken
  (56 °C mutants in laboratory), higher T do not survive
  Pasteurisation is efficient
• Sublethal heating increases the resistance to heat
• Better survival on dry foods - chocolate
Poultry as a reservoir for foodborne disease

• Poultry and **poultry products** (including **eggs** and egg products) – most significant source of human *Salmonella (Campylobacter)* infection in the developed world.

• Raw shell eggs can be contaminated by *Salmonella*, either presenting as shell contamination due to contact with faeces after laying (migration of the organism through the shell is possible) or as egg content contamination due to colonization of the hen’s oviduct.

• *Salmonella* Enteritidis Phage Type (PT) 4 high incidence since the late 1980s.
Escherichia coli

• *E. coli* belongs to family Enterobacteriaceae.
• Catalasa-positive, oxidasa-negative, facultative anaerob, fermentative, short Gram negative non sporulating rods, with peritrichous flagella.
• **Indol positive**
• Frequent formation of capsule or microcapsule
• Typical mesophilic organisms: 7 - 10ºC up to 50ºC, optimum 37 ºC.
Escherichia coli
Characteristic

• Neutral pH is optimal for growth, but has capacity to grow at pH 4.4 under optimal conditions.
• Minimal water activity for growth 0.95.
• Universal inhabitant of human and other warmblood animal gut.
• Generally harmless comensal.
• Opportunistic pathogen - infections classified as Gram negative sepsis, infections of urinary tract, pneumoniae at immunosupresive individuals and meningitides in newborn children.
Escherichia coli

Enterotoxigenic (ETEC),
Enteroinvasive (EIEC),
Enteropathogenic (EPEC),
Enterohaemorrhagic (EHEC),
Enteroaggregative (EaggEC),
Adherent *E. coli* (DAEC)
Enterotoxigenic *E. coli*

- **ETEC** - common cause of of infection diarrhoea, namely in tropic countries.
- Disease starts between **12 to 36 h** after the digestion of infectious organism.
- ETEC cause watery diarrhoea joined with convulsions, higher temperature, and nausea.
- Disease is **selflimiting**, takes **2 to 3 days**.
- Common cause of children diarrhea, serious **dehydratation**.
- Diarrhoea caused by ETEC has many common with cholera; both diseases are results of the high uptake of bacteria, that colonize small intestine, produce toxines causing the secretion to intestine lumen.
Pathogenic strains of *Escherichia coli*

- **Shiga toxin**-producing *Escherichia coli* (STEC) have been isolated from various environmental sources, such as animal faeces, food and water.
- A subgroup of STEC, the enterohaemorrhagic *E. coli* (EHEC), are serious human pathogens causing bloody diarrhoea and haemolytic uremic syndrome (HUS) mainly in children.
- The production of one or more **Shiga toxins (Stx)** is the major pathogenicity trait and responsible for the development of HUS.
- The Stx family comprises two major groups, **Stx1** and **Stx2**, sharing about 60% sequence identity.
Enteropathogenic *E. coli*

- EPEC infection cause watery diarrhoea, joined nausea and fever, is some cases long termed chronic enteritidis may occur.

- Disease starts **12-36 h** after the digestion of bacteria with food.

- EPEC is traditionally joined with outbursts in hospitals, kinder gardens and schools.

- For nursing infants and toddlers is disease more serious than many other diarrhoea infections. Can take more than **2 weeks.**
Enteroinvasive *E. coli*

- EIEC- classic symptoms of invasive bacillar dysenteria / as *Shigella* – dysentery.
- EIEC penetrates and multiply in epithelial cells of intestine, causes inflammation and ulceration.
- **EIEC strains do not produce** Shiga toxine.
- Invasivity is caused by large number of outer membrane proteins, coded by large plasmide (140 Mda).
Enterohaemorhagic *E. coli*

- Group of **EHEC** cause serious bloody diarrhoea (haemorrhagic colitide), haemolytic uremic syndrome (HUS), a thrombotic bleeding into the skin, (thrombocytopenic purpura).
- Sometimes infection cause only diarrhea without other symptoms.
- **Most frequent** EHEC strain is serotype O157:H7.
- EHEC strains produce cytotoxin **Verotoxin** (designated according its power to kill kidney cells type **Vero** - *African Green Monkey*).
German epidemic 2011

• As of June 24, 2011, 834 cases of HUS and 2,967 non-HUS cases were reported by the German Robert Koch Institute (RKI); 30 of the HUS cases and 16 of the non-HUS cases resulted in death.

• Furthermore, 100 additional infections have been identified in 12 other European countries and even in the United States and Canada (one case in Czech Republic).
German epidemic 2011

- Extensive efforts to identify the source implicated contaminated sprouts, which was later confirmed by isolation of the outbreak strain from the sprouts on June 12th.

- Historically, *E. coli* O104:H4 has been associated with very few HUS cases. To date in Germany only one of 588 EHEC strains isolated from HUS patients in the National Consulting Laboratory for HUS and the Reference Laboratory for *Enterobacteriaceae* of the RKI belongs to serotype O104:H4, and this strain was isolated in 2001.

- The *E. coli* O104:H4 isolate from this German HUS case is included in the HUS-associated *E. coli* (HUSEC) collection.
Figure 1. Events timeline of German EHEC O104:H4 outbreak.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0022751
German epidemic 2011

- On May 19, the RKI noted an increased frequency of HUS and bloody diarrhea cases in northern Germany, predominantly among adults. By retrospective analysis this outbreak had begun in early May 2011.

- The outbreak was reported for the first time to the European Center for Disease Prevention and Control (ECDC) on May 22. On May 25, the RKI and Federal Institute for Risk Assessment (BfR) issued a statement that warned against consuming cucumbers, leaf lettuce, and tomatoes, and the ECDC informed all European countries about the German EHEC outbreak, defined as such based on disease characteristics.

- One day later, STEC were detected on Spanish cucumbers by PCR, though further investigations ruled out any link to the current O104:H4 outbreak. **On June 5, sprouts were suspected** as the outbreak source by epidemiological evidence. This was confirmed by detection of EHEC O104:H4 five days later.
Figure 4. Plasmid profile of German EHEC O104:H4 outbreak strain and strain 01-09591.

Lane 1: molecular mass markers (plasmids R27 [169 kb]; R100 [90 kb]; V517 [54 kb]); lane 2: strain 01-09591 EHEC O104:H4; lane 3: German EHEC O104:H4 2011 outbreak strain LB226692; lane 4: German EHEC O104:H4 2011 outbreak strain 11-002097; lane 5: EHEC O157:H7 strain EDL 933; lane 6: E. coli 39R861 molecular size marker.

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Campylobacter

- Non-sporeforming, oxidase positive, Gram-negative rods
- Cannot ferment or oxidize sugars
- Oxygen-sensitive microaerophiles, growing best in atmosphere of 5-10% carbon dioxide and 3-5% oxygen
- All campylobacters grow at 37°C, but *C. jejuni + C. coli* optimum at 42-45°C, do not grow below 30°C.
- Principal environmental reservoir: alimentary tract of wild and domestical animals and birds, also found in rodents, dogs, cats, dairy cattle, sheep, pigs and wild birds.
Campylobacter

- Cause acute enterocolitis, not easily distinguished from illness caused by other pathogens.
- Incubation period 1 to 11 days, usually 3 – 5 days
- Fever, severe abdominal pain and diarrhoea
- Excretion of organism continues 2 – 3 weeks
- Complications – in rare cases neurological disease Guillain-Barre syndrome
- Low infectious dose /100 – 200 CFU
- In 2007 leading cause of infectious diarrhoea in Europe
Campylobacter jejuni