Enteric bacterial pathogens
Salmonella, shigella

Miklos Fuzi
Salmonella genus

- Member of the family Enterobacteriaceae
- Widespread in animals
- Gram-negative rods, capsule usually not formed
- Most variants are motile: carry the H antigen
- Composition of O antigen complex
- Lactose not fermented
- H$_2$S usually produced
- Most variants cause enteritis; two serotypes: Salmonella typhi and Salmonella paratyphi cause serious generalized infection
Grouping of salmonellae

- On the basis of genetic relatedness salmonellae can be assigned to two species:
  - S. enterica (all relevant human pathogen variants)
  - S. bongori (carried mostly by cold-blooded animals)
- S. enterica comprises of 6 subspecies (I., II. IIIa, IIIb, IV., VI., VII.)
- Human pathogenic strains belong primarily to subspecies I.
- Subspecies I comprises of about 1500 serotypes
- Official nomenclature: e.g. S. typhimurium subspecies I. typhimurium
- Name used in everyday conduct: S. typhimurium
Serotyping of salmonellae

- O antigen: shows mosaic structure; very diverse
- H antigen: also shows mosaic structure; commands two „phases”
- Typing: slide agglutination
  - O polyvalent sera – O grouping sera – O factor sera /mosaic structure entities are denoted by numbers/
  - H polyvalent sera – H factor sera (the 2nd phase can be determined on „Gard” semisolid plate containing antibodies against phase 1 H antigens)
<table>
<thead>
<tr>
<th>Serovariant</th>
<th>O antigen</th>
<th>H1 ant.</th>
<th>H2 ant.</th>
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<tbody>
<tr>
<td>S. paratyphi A</td>
<td>1,2,12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. paratyphi B</td>
<td>1,4,5,12</td>
<td>b</td>
<td>1,2</td>
</tr>
<tr>
<td>S. java</td>
<td>1,4,5,12</td>
<td>b</td>
<td>(1,2)</td>
</tr>
<tr>
<td>S. limete</td>
<td>1,4,12,27</td>
<td>b</td>
<td>1,5</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>1,4,5,12</td>
<td>i</td>
<td>1,2</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>1,9,12</td>
<td>g,m</td>
<td>-</td>
</tr>
</tbody>
</table>
Host specificity of salmonellae

- Salmonellae usually command a wide host range: infect a variety of animals apart from humans. Most important serovariants
  - S. enteritidis
  - S. typhimurium
  - S. infantis
- There are strains adapted to one particular animal species, like:
  - S. choleraesuis – pig
- Serovariants affecting exclusively humans:
  - S. typhi
  - S. paratyphi A, B, C
Salmonella gastroenteritis

- Reservoir: usually food
- Foods most often contaminated by salmonellae:
  - egg: *S. enteritidis*
  - various meat products
- Incubation: 18 - 48 hours
- Symptoms: dizziness, vomiting, stomach pain, diarrhea
- Pathogenesis: invasive infection; no exotoxin produced – salmonellae can sometime cross intestinal wall and cause bacteriaemia, sepsis
Diagnosis and therapy of salmonellosis

• Culture of pathogen on selective and/or differentiating media:
  - eosin methylene blue agar
  - bismuth-sulphite agar, Salmonella-Shigella agar
  - brilliant green agar, xylose-lysine-desoxycholate agar
  - enrichment medium (selenite bouillon)
• Molecular techniques (PCR)
• Isolated strains will be serotyped (Gard plate)
• Therapy: rehydration
  antibiotics: only in serious infections, in young children and in the elderly (extends the time of carriage subsequent infection)
• Regular checking of carriage: dependent on profession
Salmonella colonies on eosin methylene blue agar
Bismuth-sulphite agar

• Selective medium for the isolation of salmonellae
• Contains brilliant green that inhibits the growth of E. coli and the swarming of proteae
• Contains bismuth-sulphite which H$_2$S-producing salmonellae reduce to bismuth-sulfide and thus, form black colonies
Colonies of Salmonella typhimurium on bismuth-sulphite agar
Brillant green medium

- Selective and differentiating medium for the isolation of salmonellae
- Selectivity is ensured by brillant green
- Contains lactose and saccharose which if degraded turn the pH of the medium acidic. The shift in the pH is detected by the indicator Andrade (acidic fuchsin "decoloured" by alkalines)
- Salmonellae form colourless colonies; the colonies of lactose positive bacteria – some of which will grow on the medium – are red
Colonies of salmonella and E.coli on brilliant green agar
Salmonella-Shigella agar

- Selective and differentiating medium for the isolation of salmonellae and shigellae
- Selectivity is ensured by brilliant green, bile salts and Na-citrate: Gram-positive bacteria are completely inhibited, Gram-negative bacteria are partially inhibited
- The degradation of lactose is demonstrated with an indicator, the production of H₂S with the formation of iron-sulphide
- The colonies of salmonellae are black, those of lactose-positive bacteria are red
Salmonella-Shigella agar

A. E. coli  
B. Klebsiella  
C. Salmonella  
D. Proteus  
E. Pseudomonas
Stool sample on SSI medium
Xylose-Lysine-Desoxycholate agar

- Selective and differentiating medium for the isolation of salmonellae and shigellae
- Selectivity is ensured by bile acids
- The investigation of fermentative reactions is complex
- The production of \( \text{H}_2\text{S} \) is also tested
- Salmonellae form black colonies; the colonies of shigellae do not differ in colour from that of the medium; the colonies of sugar fermenters are yellow
Colonies of Salmonella typhimurium on XLD medium
Lactose positive colonies on XLD medium
Typhus abdominalis, paratyphus

- Reservoir: humans
- Transmission: contact, food
- Incubation: usually two weeks
- Pathogenesis:
  - the pathogens invade the host through the cells of the Peyer patches and infect macrophages
  - pathogens will be transferred by macrophages to the lymph nodes and various internal organs – this is called: the primary bacteriaemia
  - pathogens continue reproducing in the cells of RES – primarily in the Kupffer cells of the liver – emerge from them and disseminate throughout the body: secondary bacteriaemia
  - Long-term carriage of the pathogen does occur (gall bladder)
Typhus abdominalis

- 22 million cases/year; 200,000 deaths/year
- High continuous fever (lasts for 10-14 days without treatment)
- Malaise, backache, abdominal pain
- Headache, dizziness ("typhoid condition")
- Inproductive coughing
- Abdominal skin rashes
- Obstipation or enteritis
- Enlargement of liver and spleen
- In serious infection ileus can bleed and perforate
Diagnosis and therapy of typhoid fever and paratyphoid fever

• Isolation of pathogen from feces, blood or urine
• Serological diagnosis: tube agglutination (Widal test)
  If there is a fourfold increase in the titer between the first and the second test (10-12 days subsequent the first test) the diagnosis is confirmed
• Detection of salmonella by PCR
• Check of carriage of S. typhi after recovery; carriage can be extended, sometimes life-long
• Therapy: cephalosporins
  fluoroquinolones
  rehydration
• Prevention: strict observance of hygienic regimen; vaccine
“Central Ministry of Health in the community development areas indicated a morbidity rate varying from 102 to 2219 per 1,00,000 population in different parts of the country.

A limited study in an urban slum showed 1% of children up to 17 years of age suffer from typhoid fever every year.”

http://www.causeindia.com
Shigella genus

- Causative agents of bacterial dysentery
- Non-motile, biochemically relatively inactive, lactose not fermented
- Serotyping: on the basis of O antigen
- Most important serogroups
  - S. dysenteriae
  - S. flexneri
  - S. boydii
  - S. sonnei

All of the serogroups – with the exception of S. sonnei – comprise of multiple serotypes
Isolation and identification of shigellae

Demonstration of pathogen
- Desoxycholate-citrate agar
- Salmonella-shigella agar
- Xylose-lysine-desoxycholate agar
- No enrichment medium available
- Direct demonstration with PCR

Identification: with serotyping sera
Desoxycholate-citrate agar (DC)

• Selective and differentiating medium for the isolation of shigellae

• The selective agent is bile salt, differentiation is achieved with lactose and neutral red indicator

• Coliform bacteria – if grow on the medium – form red colonies; the colour of the shigella colonies resemble that of the medium
Colonies of shigella on DC medium
Colonies of shigella on XLD agar
The pathogenesis of shigellosis

• Shigellae are highly invasive pathogens (with the exception of S. sonnei) capable of invading intestinal epithelial cells and propagating in them. This characteristic is coded on a plasmid and expression is governed by temperature: at or above 35°C shigella strains are particularly invasive.

• S. dysenteriae: causes large outbreaks with serious infections. Produces „Shiga toxin” – closely related to the verotoxin of EHEC – capable of eliciting haemolytic uremic syndrome (HUS).

• S. sonnei: less invasive serogroup.
The epidemiology of shigellosis

- Obligate human pathogen
- About 18,000 cases reported annually in the US
- Transmission: very easy – a few bacteria are capable of eliciting shigellosis
  - contact (in areas lacking running water)
  - water (including swimming pools)
  - food
- In industrialized countries S. sonnei in developing countries S. dysenteriae and S. flexneri are prevalent
- Shigellosis is endemic in many developing countries
- S. sonnei is less likely to cause outbreaks and infection is associated with milder symptoms
The symptoms of shigellosis

- Bloody diarrhea (dysentery)
- Vomiting
- Abdominal cramps
- Malaise
- Fever
- Dehydration
- Seizure (mainly in children)
- Haemolytic uremic syndrome (in infections with S. dysenteriae)
- Postinfectious reactive autoimmune arthritis
Therapy and prevention of shigellosis

- Therapy: antibiotics should always be given
  - trimethoprim/sulphamethoxazole
  - 3rd generation cephalosporins
  - fluoroquinolons

- Rehydration

- Prevention: Strict observance of hygienic rules

- Immunity: type specific immunity develops after infection (unlike in most other enteric infections)
Shigellosis - WHO

"Shigellosis is endemic throughout the world where it is held responsible for some 120 million cases of severe dysentery with blood and mucus in the stools, the overwhelming majority of which occur in developing countries and involve children less than five years of age.

About 1.1 million people were estimated to die from Shigella infection each year, with 60% of the deaths occurring in children under 5 years of age. "
During the 1994 genocide in Rwanda, approximately 20,000 Rwandan refugees who had fled into the North Kivu region of Zaire died in the first month alone from dysentery caused by a strain of Shigella that was resistant to all commonly used antibiotics.

The combination of Shigella and HIV infections has deleterious consequences, due to compromised immunity in HIV-positive persons.
Thank you for your attention