15. Enterobacteria & enteric pathogens

15.1. GENERAL FEATURES

Enterobacteria are gram-negative predominantly motile rods naturally found in the intestinal tracts of humans and animals where they are a part of the commensal flora. However some are serious human pathogens of of gastrointestinal tract - GIT (e.g. genera *Salmonella, Shigella,* virotypes of *Escherichia coli*). Enteric pathogens like *Campylobacter* and *Helicobacter*, although not a part of the *Enterobacteriaceae*, also have the capacity to cause serious infection of GIT.

15.2. VIRULENCE FACTORS & PATHOGENESIS

Virulence factors are genetic, biochemical, or structural features that enable an organism to cause disease.

The capsule (usually made of polysaccharides) causes attachment (e.g. *E.coli* to the epithelial cells of GIT or urinary tract) or has antiphagocytic or **serum resistance** activity (e.g. in *Klebsiella* infections). **Fimbriae** are proteinaceous hair-like structures responsible for the specific attachment to host cell receptors (e.g. enterotoxigenic *E.coli*). **Invasion proteins** rearranges the actin filaments of the host cell with subsequent engulfment of the salmonellae, leading to intracellular replication in the phagosome with eventual host cell death and spread to adjacent epithelial cells and lymphoid tissue (e.g. salmonella secreted invasion protein *Sips*). **Endotoxin (LPS - lipopolysaccharide)** liberated from the cell wall when gram-negative bacteria lyse interact with macrophages that as a response release cytokines and activates complement and coagulation cascades (consequences are peripheral vasodilatation with hypotension, fever, leucopenia, disseminated intravascular coagulation, shock etc.). **Antigens and phase variation:** Serotypes are characterised by somatic (O) (part of LPS), flagellar (H) or capsular (K) antigens. The phase variation means that these antigens can be alternately expressed or not expressed at all. This protects the bacteria from antibody-mediated cell death and therefore makes them more virulent.

Cytotoxins (shiga toxin, verotoxin) and enterotoxins: Heat-stabile (ST) and heat-labile (LT) enterotoxins are produced by a small variety of bacteria, notably by enterotoxigenic *E.coli* (ETEC). LT is a bi-component exotoxin. It activates adenylyl cyclase and therefore increases intracellular cAMP production, which causes hypersecretion of water and chlorides. ST activates guanylyl cyclase in enteric epithelium, which leads to excessive fluid secretion. *Helicobacter* produce **ammonia**, which protects the bacteria from the acidic stomach juice.

15.3. INFECTIONS & EPIDEMIOLOGY

Gastrointstinal Infections. *Salmonella, Shigella and virotypes of E. coli* commonly cause gastrointestinal infections. Infection is spread via the fecal oral route or from contaminated raw foods. Symptoms usually appear after a few days (e.g. 48h in salmonella GIT) when the cause is bacterial. The symptoms appear much sooner after toxic insults. The disease may present itself with vomiting and stomach cramps or diarrhoea. This is dependent upon the causative agent (e.g. enterotoxic, enteroinvasive, enteropathogenic, enteroaggregative or enterohemoragic forms of *E.coli* etc.)

Extraintestinal infections are usually urinary tract infections or wound infections and often occur as hospital acquired infections (nosocomial). Systemic infections can, in some

cases, arise from *Salmonella, Shigella* and *Escherichia*. For example, *Salmonella* Typhi can cause bacteriema. It does so by penetrating the small bowel and multiplying in the intestinal lymphoid tissue before entering the blood stream.

15.4. TREATMENT, PREVENTION & CONTROL

Gastrointestinal infections: water and ion replacement is essential to rehydrate the patients. Prescription of an antibiotic therapy is controversial because it may prolong the convalescent carrier state. Exceptions exist in the cases where the agent crosses the intestinal barrier and enters the host (shigellosis). In case of helicobacter infection combination therapy (proton pump inhibitor and antibiotics) are used.

Extraintestinal infections are treated with antibiotics against the agents. Because of quickly acquired chromosomal or plasmid-mediated resistance to antibiotics the activity of the drugs should be tested in-vitro before treatment. Treatments of choice are the beta-lactams (e.g. ampicillin), sulphonamides (e.g. trimetoprim and suphamethoxazol), fluorochinolones, aminoglycosides and 3rd generation cephalosporins. In the case of systemic infection of Campylobacter macrolides could also be used.

15.5. LABORATORY DIAGNOSIS

<u>The presumptive decision to treat shigellosis must be based on clinical observation and must not be delayed!</u>

a) Specimen collection: stool, anal swabs, and other clinical material depending upon the localization of the infection.

b) Microscopy: with exception of *Helicobacter* and stomach biopsies (fig. 1A), microscopy from direct clinical material is relevant only in case of extraintestinal infections (fig. 1B, 1C).

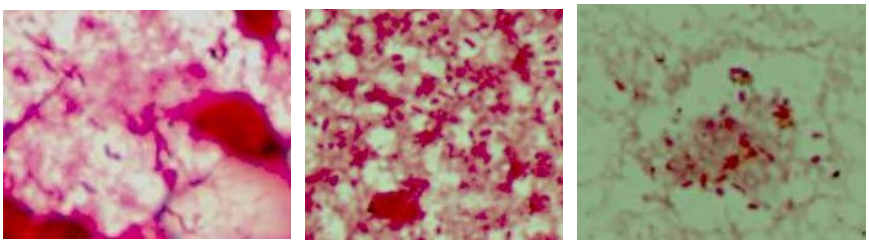


Fig.1. Helicobacter pylori in stomach biopsy (rods or „flying birds“)(A), Gram-negative rods in hemoculture of a febrile patient (B), Gram-negative rods and tissue debris from an abscess (C)(Klebsiella pneumoniae was cultivated from the patients B and C)

c) Culture: Enterobacteriae and vibrio grow rapidly (overnight) on general media (nutrient agar) commonly as grey colonies. Blood potentiates the growth and hemolysis can be seen in some strains around the colonies (e.g. uropathogenic *Escherichia coli*). Stool or anal swabs are inoculated on selective or diagnostic media to eliminate growth of competitive flora (e.g. deoxycholate and Endo agar) (fig.2).

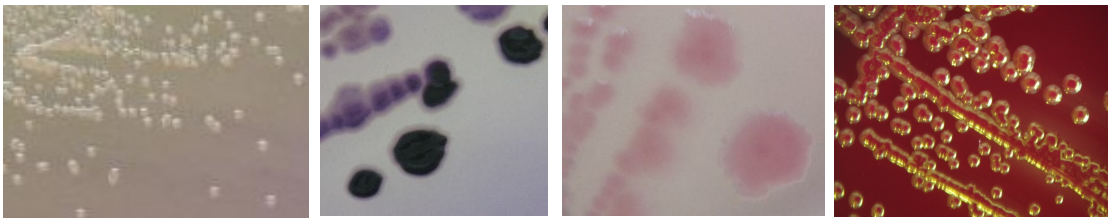


Fig. 2. A diagnostic media for lactose fermenting (usually commensals) and non-fermenting enterobacteriae (usually enteropathogenic). Yersinia enterocolitica growing on deoxycholate agar (A), Black colonies of Salmonella sp. producing hydrogen sulphide on MLCB agar (B) and does not ferment lactose on Endo agar (C), Escherichia coli fermenting glucose on Endo agar with the typical metal gloss known for coliform bacteria (D)

d) Phenotypical identification is focused on detection of specific biological properties of the agents (microscopy, bacterial colonies, enzyme detection) (see also chapter – Identification). This could be used in screening tests (preliminary identification). Some steps are demonstrated in fig. 3.

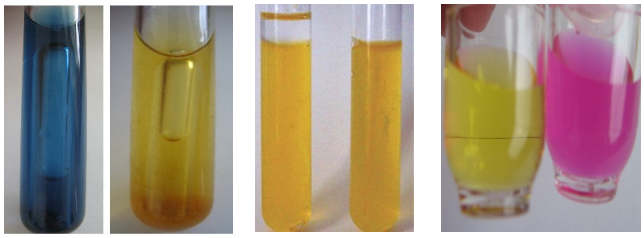


Fig.3. A. Enterobacteria ferment glucose with gas production (B), negative control (A) and also under minerail oil (C), oxidation of glucose (D). Production of ammonia from a stomach biopsy of a patient infected by Helicobacter pylori is indicated by red colour (F), negative control (E)

Biochemical identification. Multiple enzyme tests are performed to identify the bacteria. Positive and negative reactions are noted. The metabolic profiles of known species are compared with the strain being analysed. Key dichotomous method and numerical identification are used (see also chapter 6 – Identification).

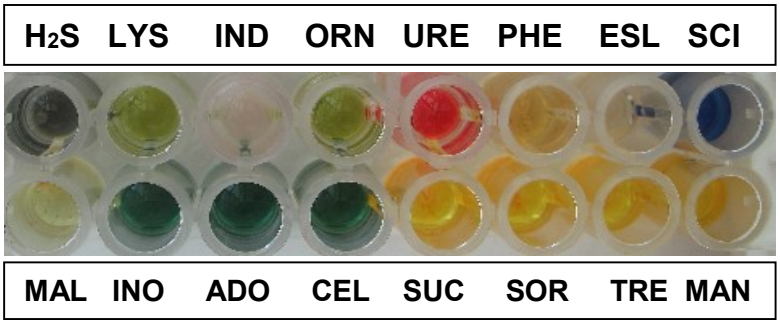


Fig. 4. Biochemichal profile of a clinical isolate tested using set of substrates

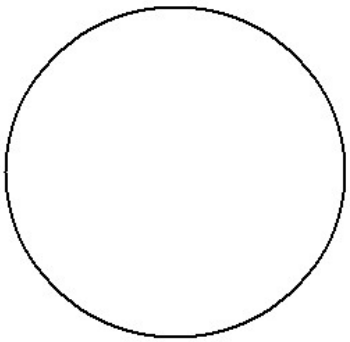
Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry - (MALDI – TOF MS): analyses molecular structure (mainly proteins) of an unknown microbial isolate, as the mass spectral pattern consisting of a number of structurally related mass spectral peaks, and comparing with a known patterns analyzing the isolate. The mass spectrometer first ionizes, then mass separates and finally detects time of the ions flight, thus producing a mass spektrum and comparing it with known mass spektra analyse the studied microorganism (see also chapter 6 - Identification).

Identification by serotyping (reverse agglutination) uses specific O, H and K antisera for significant pathogens (e.g. Salmonella) or pathogenic serotypes for identification (e.g. virotypes of *E.coli*).

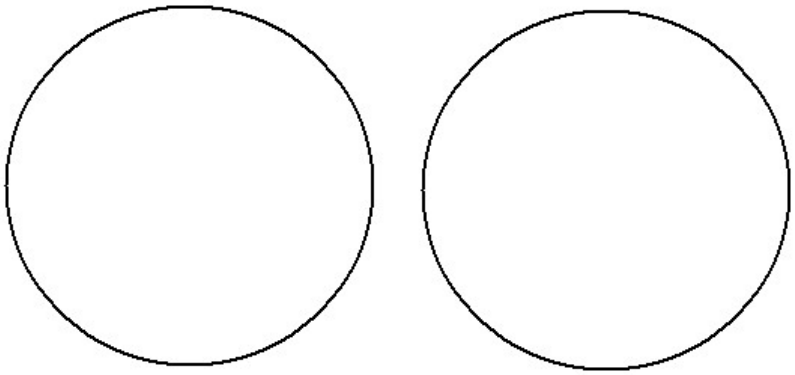
e) Genotypical identification. There are several ways to identify a strain or species using genotype. For instance, comparing electrophoresed fragments allows visualization of the restriction profile. Alternatively, homology of highly conserved regions, such as the 16S RNA gene, can assist identification of the species (see also chapter 6 – Identification).
f) Susceptibility testing. Qualitative (disk diffusion method), quantitative methods or their combination (E-test) are used to test the susceptibility (see also chapter 5 – Antibiotic susceptibility testing).
g) Serological diagnosis (Widal reactions): sera of suspected patients with *S. typhi* or *S. paratyphi* infections (enteric fever) are analysed using somatic (O) and flagellar (H) antigens.

15.6. PRACTICAL PART – ENTEROBACTERIA AND ENTERIC PATHOGENS

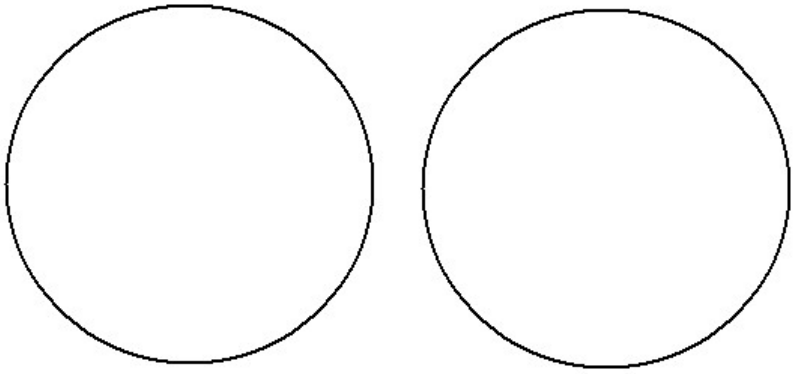
Exercise 1: MICROSCOPY: prepare a gram-stain of a smear from a hemoculture of a febrile patient. After drying, fixing and gram-staining draw the morphology of the colony that you could see in your microscope.



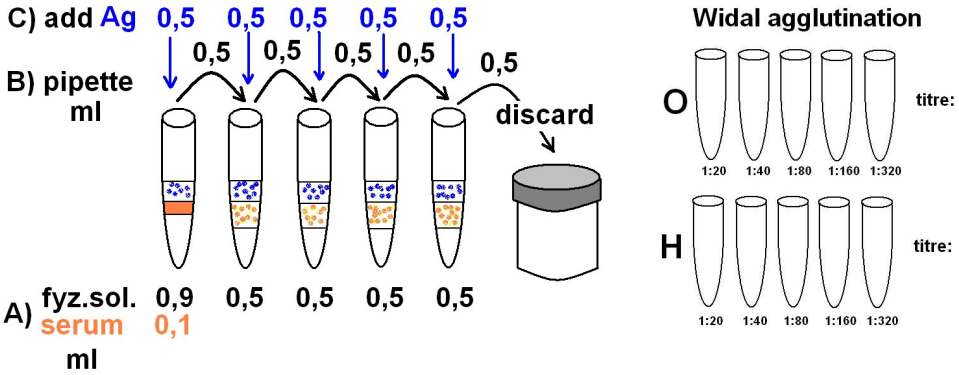
Exercise 2: MICROSCOPY: Stain colonies of an enterobacterial species growing on culture media. After drying, fixing and gram-staining draw the morphology of the colony that you can see in your microscope.



Exercise 3: SEROTYPING (REVERSE AGGLUTINATION) OF ENTEROPATOGENIC E.COLI O55. Mix the culture of *E. coli* of an infant with antiserum against O55 serotype. Positive agglutination will appear if the analysed strains is of the O55 serotype. Draw the reaction.



Exercise 4: *INTERPRET THE RESULT OF SEROLOGICAL ANALYSIS OF A PATIENT WITH SUSPECTED ENTERIC FEVER. Dilute the serum of a patient geometrically, add the H and O Ag (following steps A, B and C indicated in the schema below), incubate overnight at 37°C and 45°C, respectively. Observe the positive reaction and determine the specific antibody titer (see also chapter 7 - Serology). Patient sera are diluted geometrically: 1:20, 1:40, 1:80, 1:160 and 1:320*



14.8. LAB QUIZ

1. Specify some virulence factors of the enterobacteria.
2. Specify two categories of infections caused by the enterobacteria.
3. Describe principles of treatment of enteric infections.
4. Specify direct methods to diagnose enterobacteria and enteric pathogens.
5. Specify prevention and control of the infections.