

Enterobacteria Diagnostics,
cultivation, identification
(biochemistry, agglutination),
clinical significance

Enterobacteria

- Families: Enterobacteriaceae, Morganellaceae, Yersiniaceae
- located in the gastrointestinal tract of humans and animals
- facultative anaerobic gram-negative rods
- non-sporulating
- growth on the basic media
- ferment glucose
- antigenic structure:

cell wall structure: lipopolysaccharide , includes somatic **antigen „O“** - some of them motile, the flagella are built of protein – **„H“ antigens** - may have a capsule - capsular **K antigens**, *Salmonella typhi* – **„Vi“ antigen** (virulence Ag)

Family Enterobacteriaceae

- contains more than 30 genera (genus) about 100 species (species)
- Obligate pathogens:
 - *Salmonella*
 - *Shigella*
 - Primary pathogenic *E. coli*
- Opportunistic pathogens: Escherichia, Klebsiella, Enterobacter, Citrobacter

The clinical significance

- Obligate pathogens:
 - The causative agents of foodborne diseases
- Opportunistic pathogens:
 - Most of them are part of the intestinal microflora
 - The causative agents of urinary tract infections, pneumonia, sepsis, wound infections, meningitis...

Laboratory diagnosis

Direct detection

- **Microscopy:** Gram staining
- **Cultivation:** primary and selective culture media subsequent identification - biochemical tests, mass spectrometry (MALDI-TOF)
- **Serotyping:** more detailed analysis using agglutination → serotypes
- **PCR:** Detection of specific agents (i.e. *E.coli* in the CSF) Multiplex PCR – i.e. for detection of enteric pathogens

Indirect methods (serology)

- detection of antibodies in the blood serum
- Widal reaction – agglutination in tube, dg. of typhoid fever by means of O, H, Vi

Genus: Salmonella

- Most important subspecies: *S. enterica ssp. enterica*, in the human medicine
 - It is distinguished more than 2500 serotypes - by antigenic structures O and H Ag
 - significant serotypes:
 - *S. enterica ssp. enterica* serotype Typhi (It is used commonly abbreviated title *Salmonella* Typhi)
 - *Salmonella* Enteritidis
 - *Salmonella* Typhimurium

Genus Salmonella

- **Biochemistry:** does not utilize lactose, hydrogen sulfide production
- **Cultivation** on the selective media:
 - Endo agar, MacConkey agar
- grows in light transparent colonies = lactose – negative on desoxycholate citrate agar in light colonies with black dot in the center of the colony

Genus: *Salmonella* – antigenic structure

- more than 2500 serotypes – detailed identification into serotypes - for epidemiological reasons
- Kaufmann-White's scheme – for detailed identification of *Salmonella*
- different species may be distinguished by somatic O antigens (Ag) (expressed in figures), according to them, are divided into groups
- *Salmonella* within a group are different according to flagellar H Ag (small letter designation and number)
- Some salmonella have a capsular Ag– i.e. *Salmonella* Typhi has Vi Ag

Genus *Salmonella* – pathogenicity

- Anthropopathogenic – *S. Typhi*, *S. Paratyphi A*, *B*, *C* – Causative agents of typhoid and paratyphoid fever – The source of infection - only human beings (sick person, carrier)
 - Incubation period: 10 – 14 days
 - Severe systemic disease, characterized mainly by fever, headache. Involvement of the gastrointestinal tract has the characteristic symptoms (constipation, diarrhea),
- Primary zoonopathogenic – *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Hadar*...
 - Causative agents of gastroenteritis
 - Source of infection: poultry, other domestic livestock, birds, snakes, etc. vehicle = contaminated food
 - interhuman transmission is rare

Laboratory diagnosis

- Salmonellosis – Cultivation of rectal swab
- Typhoid fever
 - Serology diagnosis: Widal's reaction: antibody detection
 - Cultivation:
 - hemoculture
 - anal swab
 - Urine

Genus *Shigella*

- more sensitive than other intestinal rods to environment, not found as a part of environment
- pathogenic for humans,
- the infectious dose is relatively low
- attack colonic mucosa causing bacillary dysentery (dysentery), production of shigatoxins
- transmitted directly from person to person, or by contaminated water
- Epidemiology - in children's communities, hospitals and social facilities

Genus *Shigella*

- *Shigella* sp. - genetically are similar to E.coli
- There are 4 types of *Shigella*:
 - *S. dysenteriae*
 - *S. sonnei* – the most common in the Czech Rep.
 - *S. flexneri*
 - *S. boydii*

Genus *Shigella*

- biochemical activity: lactose negative
- **Cultivation:**
- on the selective media:
 - Endo agar, MacConkey agar, - they grow in colourless colonies
 - DC – they grow in pink flat colonies
- **Laboratory detection:** – Cultivation of the rectal swab collected into transport culture medium

Genus *Escherichia*

- a part of the microflora of the colon of animals and humans
- Function of the normal microflora: to prevent attachment of enteric pathogens vitamin K and B formation
- Pathogenic effects:
- Outside the intestine (GIT) can cause inflammation - in the urinary tract, intra-abdominal infections ..
- Some strains are pathogenic for intestine
- The most important representative : *E. coli* – rarely can be demonstrated in humans *E. hermannii*, *E. vulneris* – according to Ag structures we can distinguish 240 serovars

Pathogenesis

- Virulence factors:
- Adhesins
 - colonization factors (for attachment of enteropathogenic strains of *E. coli* on the intestinal epithelial cells)
 - P-fimbriae – can bind to the epithelial cells
- Toxins
 - Endotoxin
 - Exotoxins – thermolabile and thermostable, shigatoxin
 - hemolysins

Pathogenicity

- **Primary pathogen**

- *Intestinal infections* (diarrheagenic *E. coli*):

- Pathogenic effects on the intestine
 - prerequisite for adhesion to the intestinal mucosa
 - ETEC – enterotoxigenic *E. coli*
 - EIEC – enteroinvasive *E. coli*
 - STEC (VTEC) – strains – producers of shiga-toxin (verotoxin)
 - EHEC – enterohaemorrhagic *E. coli*
 - EPEC – enteropathogenic *E. coli*
 - EAGEC - enteroaggregative *E. coli*

- *Extraintestinal infections* (ExPEC):

- Uropathogenic *E. coli* (UPEC) Urinary tract infections – 80% of community infections
 - uropathogenic strains of *E. coli* with adhesins ascending route of infection (strains colonize the intestine) from the external urethral orifice, resist the flow of urine get into higher parts → cystitis and pyelonephritis
 - Neonatal meningitis/sepsis MNEC – strains bearing K1 capsule

- **Opportunistic pathogens:**

- catheter-related uroinfections;
 - Intra-abdominal infections – Cholecystitis, cholangitis;
 - sepsis

Intestinal infections - pathogenesis

- **ETEC – enterotoxigenic *E. coli***
 - occurrence – belt of the tropics and subtropics
 - production of thermolabile and thermostable enterotoxin (similar to *Vibrio cholerae* enterotoxin), affects the small intestine
 - the most frequent cause of traveller's diarrhea
- **EPEC – enteropathogenic *E. coli***
 - the ability to adhere to the enterocytes in the small intestine, destroy the microvilli of enterocytes on their surface
 - cause of watery diarrhea among the youngest children, epidemics in neonatal units
 - Most frequent serotypes: O26, O55, O86, O111, O124-128
- **EIEC – enteroinvasive *E. coli***
 - It occurs mainly in developing countries
 - like shigella these strains are not limited to adhesion, but penetrate into the colon cells and multiply in them

Intestinal infections - pathogenesis

- **STEC (VTEC) – shigatoxin producers**
 - Production of shigatoxin (similar to *Shigella dysenteriae*)
 - destroy proteosynthetic apparatus of enterocytes
 - Mostly affects children and the elderly
 - Sporadic minor epidemic
- **EHEC – enterohemorrhagic *E. coli***
 - is a subtype of STEC, causes severe clinical course
 - reservoir – calves, sheep, goats
 - source of infection: ingestion of undercooked meat, contaminated water, unpasteurised milk
 - Most frequent serotypes: O157:H7, O26, O103 – O157:H7
 - cultured on MacConkey agar with sorbitol (O157:H7 is sorbitol negative and grows in light colonies)
 - complication: Hemolytic uremic syndrome (HUS) - fatal

Genus *Yersinia*

- causative agent of plague - *Yersinia pestis*
 - Still occurs in natural foci in Asia and Africa
- virulence factors – capsule (prevents phagocytosis), endotoxin, invasin (it enables the rapid penetration through the tissues)
- Source of infection – rats, ... contagion – oriental rat flea (*Xenopsylla cheopis*)
- Entry into the body – skin, lungs

Genus *Yersinia*

- Causative agent of intestinal infection – *Y. enterocolitica*
- Complications: enterocolitis with high fever and abdominal pain (“false appendicitis”), possible extra-intestinal complications (i.e. arthritis, erythema nodosum)
- The source of the infectious agent: animal - hog (pig), various domestic and wild animals
- Laboratory detection:
 - Cultivation of the anal swab – CIN agar

Other enterobacteria

- Genus *Klebsiella*
 - species: *K. pneumoniae*, *K. oxytoca* – colonize the GIT (gastrointestinal tract)
 - The causative agents of urinary tract infections *K. pneumoniae* - the second most common Gram negative rod after *E. coli* → pneumonia, sepsis (frequently nosocomial)
 - most infections are endogenous
 - important virulence factor: the presence of the capsule
 - *K. pneumoniae* – frequent ESBL producer
- Genus Enterobacter – *E. cloacae*, *E. aerogenes*
 - part of the normal intestinal microflora
 - Causes similar infections as *Klebsiella*
 - Hospital strains are often producers of AmpC or ESBL

Other enterobacteria

- Genus *Citrobacter* – *C. freundii*, *C. koseri*
 - Part of the intestinal microflora, causative agent of urinary tract infections
- Genus *Serratia* - *S. marcescens* (producer of a red pigment - a hospital pathogen, relatively resistant to disinfectants and antibiotics)
- Genera: *Proteus*, *Morganella*, *Providencia*
 - Part of the intestinal microflora, agents of urinary tract infections
 - Highly biochemically active – utilize protein and urea
 - *P. mirabilis* and *P. vulgaris* – Creeping grow on agar surface (swarms)
 - Participate in the formation of kidney concrements – decomposition of urea to (amonia and CO₂) increasing the pH of urine – precipitation of calcium and magnesium ions.
 - Secondarily colonize chronic defects