Theoretical Section – What Every Student Should Know:

1) The intestinal microbiota – its role in human health, with relevant examples.
2) Defense mechanisms of the gastrointestinal tract – list them and explain their role in protecting the GIT.
3) Mechanisms of food poisoning caused by bacterial enterotoxins – provide examples of intoxications, the toxins involved, and their sources and reservoirs. Should foodborne intoxications be treated? If so, how? Should they be diagnosed – and by what methods?
4) Botulinum neurotoxin poisoning – mechanism of toxin action, pathways of entry into the human body, and management of botulism, including diagnosis, treatment, and prevention.
5) Bacterial enterotoxins – characteristics, mechanisms of action, and examples.
6) Bacterial cytotoxins – characteristics, mechanisms of action, and examples.
7) Invasive vs. non-invasive gastrointestinal infections (GITIs) – how they differ, with examples.
8) Impact of infectious dose and pathogen virulence on the development and course of GIT infections. Please provide examples of infections with low contagious doses and describe their transmission routes.
9) Epidemiology of GIT infections – identify which infections are primarily foodborne, which spread via the fecal–oral route, and which may originate from environmental sources.
10) Selected gastrointestinal infections – etiological agent, predominant symptoms and complications, diagnosis, and treatment: yersiniosis, campylobacteriosis, shigellosis, salmonellosis, typhoid fever, listeriosis, and vibriosis.
11) Viral gastrointestinal infections – causative viruses, general clinical course, complications, diagnosis, and treatment.
12) Treatment of GIT infections – antibiotics used empirically and in targeted therapy. What resistance mechanisms may enteropathogens exhibit?
13) Drugs of choice in the treatment of GIT infections caused by pathogenic E. coli, Salmonella, Shigella, Campylobacter, Yersinia, Helicobacter, and C. difficile.
14) Diagnosis of GIT infections – in which conditions do culture, molecular tests, and serology have the most significant importance? Provide examples and justify.
15) Types of specimens for diagnostic testing, transport conditions, and directions for laboratory investigations.
16) Diagnosis of infections caused by H. pylori and C. difficile.
17) Specific and nonspecific prevention of GIT infections.

Diagnosis of gastrointestinal infections most commonly concerns cases that:
a) pose a threat to the patient's health or life (e.g., profuse diarrhea leading to rapid dehydration in infants and the elderly, or foodborne intoxication caused by Clostridium botulinum),
b) are caused by invasive pathogens that damage the intestinal epithelium and lead to ulceration,
c) may progress to systemic infections (e.g., typhoid fever) or result in serious extraintestinal complications such as Reiter's syndrome or Guillain–Barré syndrome,
d) are of significant epidemiological importance and may pose a public health concern (e.g., outbreaks, epidemics, or pandemics).

DIAGNOSIS OF FOOD POISONING

Diagnostic testing is performed only very rarely (primarily for epidemiological purposes), due to the instability of bacterial toxins and their typically low concentrations in contaminated food. Moreover, the characteristic clinical presentation of botulism eliminates the need for routine microbiological diagnostics. At the same time, food poisoning caused by staphylococcal enterotoxins or toxins produced by Bacillus cereus usually resolves spontaneously without treatment and seldom prompts patients to seek medical attention. In the context of foodborne outbreaks, the material submitted for bacteriological examination is typically the contaminated food that served as the source of poisoning, and less frequently, stool or vomit samples, which may be analyzed for the presence of known bacterial toxins.

DIAGNOSIS OF GASTROINTESTINAL INFECTIONS

The diagnosis of gastrointestinal infections involves integrating clinical findings with microbiological investigations. Laboratory testing is particularly relevant in cases with severe or systemic manifestations, in patients at high risk of complications, or when infections are of epidemiological concern. The diagnostic approach typically includes stool analysis, microbiological culture, molecular testing, and serological assays, which are selected according to the suspected pathogen and the clinical presentation. Appropriate sampling and

transport conditions are essential to ensure reliable results, and diagnostic outcomes must always be interpreted in the context of the patient's symptoms and epidemiological background.

Detection of the causative pathogen in diarrheal disease is not always necessary, and most cases of secretory diarrhea are not routinely investigated. This is due to several factors: the large variety of bacterial species capable of producing enterotoxins (including members of the normal intestinal flora that may acquire enterotoxin genes in vivo), the self-limiting nature of the majority of secretory diarrheal episodes, and their typically mild clinical course.

Indications for microbiological diagnosis include: severe forms of gastrointestinal infection (GII), particularly those presenting with blood in the stool and/or systemic manifestations, hospital-acquired gastrointestinal diseases, and chronic or recurrent infections.

Follow-up testing is not performed after clinical symptoms of infection have resolved, whether spontaneously or following treatment.

Specimen collection:
a) Stool samples
b) Rectal swabs, used exclusively for epidemiological investigations of carrier status for Shigella and Salmonella
c) In systemic gastrointestinal infections (GII), blood and other biological materials, such as bone marrow or urine, may be collected—for example, in patients with typhoid fever.

Diagnostic methods depend on the suspected etiology of infection and include:
1. Culture and identification – differential and selective media for Salmonella/Shigella (SS), Gram-negative bacilli (MacConkey, MC), and chromogenic media for the detection of specific pathogens (e.g., EHEC, Shigella, Salmonella, Candida). Culture remains the routine diagnostic method in most laboratories worldwide. Identification of pathogens is based on their biochemical properties. Selected groups of pathogens, for which biochemical identification is limited to the genus level, require species-level assignment through serotyping. This applies particularly to Salmonella and Shigella, where classification is based on somatic (O) and flagellar (H) antigens. Serotyping is also used for the identification of enteropathogenic E. coli (EPEC).
2. Antibiotic susceptibility testing, especially for pathogens with increasing resistance, such as Campylobacter, Salmonella, and Helicobacter.
3. Rapid tests, which detect antigens of Salmonella, Shigella, Campylobacter, Yersinia, C. difficile, EHEC, and most viral agents responsible for diarrhea. Rapid tests may also detect important markers of intestinal inflammation, such as calprotectin and lactoferrin**.**
4. Molecular tests (e.g., PCR), which identify genetic material of selected pathogens, including viruses or toxin-encoding genes. These are typically used for epidemiological purposes, in diagnostically challenging cases, and routinely as the "gold standard" in yersiniosis.
5. Microscopy, applied only as a supportive method to assess bloody diarrhea (presence of erythrocytes) and intestinal inflammation (presence of leukocytes).
6. Serology, detecting pathogen-specific antibodies in selected infections such as yersiniosis.

Diagnosis of Helicobacter pylori Infections
1. Non-Invasive Methods

a) Detection of H. pylori antigens in stool samples using commercially available cassette tests.
b) Urea breath test – the patient ingests orange juice containing urea labeled with a radioactive carbon isotope. H. pylori present in the stomach produces a high level of urease, which hydrolyzes the urea into water and carbon dioxide containing the radioactive carbon isotope, subsequently measured in the exhaled air.

2. Invasive Methods
Endoscopic biopsy samples from the gastric mucosa may be obtained for the following tests:

a) Urease test – confirms the presence of H. pylori (only H. pylori produces high levels of urease within minutes to an hour). The biopsy specimen is placed in a medium containing urea and a pH indicator and incubated at 37 °C. A color change of the indicator, typically observed within 30 minutes, indicates alkalinization of the medium and, consequently, urease activity.

b) Histopathology – staining with histological techniques or Gram staining allows visualization of H. pylori within the gastric mucosa and assessment of key parameters: density of colonization and degree of inflammatory changes.

c) Culture of H. pylori – performed on special blood-enriched culture media to confirm species identity and determine antibiotic susceptibility. This is of clinical importance due to the increasing resistance of H. pylori to antibiotics. Culture remains the so-called "gold standard" in the diagnosis of H. pylori infection.

d) PCR testing – for the detection of H. pylori and its toxin-encoding genes.

Diagnosis of Clostridioides difficile Infections

Specimen for Testing: **diarrheal (liquid) stool**

a) **Detection of glutamate dehydrogenase (GDH).** Both toxigenic and non-toxigenic strains produce this enzyme. Screening for GDH allows for the rapid exclusion of infection.

b) **Detection of toxins**: (a) enzyme immunoassays (EIA); (b) molecular tests detecting genes encoding toxins.

c) **Culture and biochemical differentiation of microorganisms** isolated from stool samples, followed by confirmation of toxigenic strains through toxin detection in cell culture assays.

d) **Microscopy** – histopathological examination of colonic biopsy specimens.

PRACTICAL SECTION

CULTURE OF GASTROINTESTINAL TRACT SPECIMENS

Case 1.

A 19-year-old graduate of a culinary technical school is applying for a job at a fast-food restaurant. Her application was accepted, but before starting work, she was required to undergo microbiological (and parasitological) stool testing for carriage of gastrointestinal pathogens. For carrier screening, three stool samples are collected on three consecutive days. During laboratory classes, students culture one stool specimen obtained with a swab on MacConkey (MC) and Salmonella-Shigella (SS) agar.

Questions for Students:

1. Assess the colony morphology on MC agar: ……
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2. What is necessary to classify the grown bacteria to the species level? ……
3. How can the isolated bacteria be identified? ……
4. Based on bacterial growth characteristics, which organisms could have grown from the culture?
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5. What does the isolation of these bacteria indicate for the tested individual? ……
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6. Is pathogen isolation in this case an indication for antibiotic therapy? ……
7. Which antibiotics are effective against infections caused by these pathogens, and when should such infections be treated? ……
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Case 2.

An 18-month-old male child was admitted to the Department of Pediatrics, Gastroenterology, and Nutrition due to a five-day history of watery diarrhea, crampy abdominal pain, and vomiting. Clinical examination revealed dehydration and weight loss. Stool analysis showed the presence of mucus, but no blood. Initial testing excluded viral etiology — antigen assays for Rotavirus and Adenovirus were negative. A stool sample was collected for bacteriological testing. During the laboratory session, students inoculate the specimen obtained with a swab onto MacConkey (MC) and Salmonella–Shigella (SS) agar.

Questions for Students:

1. Evaluate the colony morphology on MacConkey agar, lactose-fermenting colonies appear pink; on Salmonella–Shigella agar: ……
2. What is required to classify the isolated bacteria to the species or possibly serotype level? ……
3. How can the isolated bacteria be identified? ……..
4. Based on growth characteristics, which bacteria are most likely isolated from the culture? ……..
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5. Does isolation of these bacteria indicate the etiologic agent of the infection? ……
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6. How should this infection be treated in the child? ……
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7. Is antibiotic therapy necessary? ……
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8. Is it necessary to confirm eradication of the pathogen with a follow-up stool culture? ……

Case 3.

An 80-year-old female patient was admitted to the Department of Anesthesiology and Intensive Care due to acute respiratory distress syndrome (ARDS). On the third day of hospitalization, she was diagnosed with microbiologically confirmed pneumonia caused by Acinetobacter baumannii. Antibiotic therapy was initiated according to the susceptibility profile (ampicillin–sulbactam).

After one week of treatment, the patient developed watery diarrhea (up to 10 stools per day with mucus), nausea, and lower abdominal pain. On physical examination, signs of dehydration, abdominal distension, and tenderness on palpation were observed. A stool sample was collected for testing.

Questions for Students:

1. What is the most probable diagnosis in this patient? Justify your answer.
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2. What are the risk factors for developing diarrhea during antibiotic therapy? ……..
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3. How should such diarrhea be treated? ……
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4. What does the isolation of this pathogen mean for the patient? For the hospital? Does it require any action?
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Case 4.

In February 2023, a five-year-old boy was brought to the emergency department in severe general condition. History obtained from the child's parents revealed that he had been experiencing vomiting and watery diarrhea (on average, five loose stools per day) for the past three days, accompanied by fever up to 39 °C. Physical examination demonstrated dehydration, tachypnea, dry oral mucous membranes, and hyperemic palatine arches.

Questions for Students:

1. What is the most probable diagnosis, considering the child's age and the season?
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2. How should this infection be treated? ……
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3. How could the child have become infected? ……
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4. Could this infection have been prevented? ……
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5. Are infections caused by this pathogen associated with complications? If so, give examples……
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Clinical cases for class discussion:

1. Infection with Yersinia enterocolitica – clinical course depending on the age of the infected individual

A father and his children spent their vacation at their grandparents' farm. The family consumed raw cow's milk daily as well as meat dishes prepared from home-slaughtered animals. The children (a 5-year-old boy and a 12-year-old girl) eagerly played with the animals (direct contact – petting, feeding). Between days 7 and 10 of their stay, all family members (except the grandparents) developed illness, though the clinical manifestations differed:

a) In the boy, watery–mucous diarrhea with small streaks of blood, low-grade fever, and diffuse abdominal pain occurred. Without treatment, the symptoms gradually resolved within a week.

b) In the girl, the initial clinical symptoms resembled those of her brother but significantly worsened after another 3 days, when the pain localized in the right lower quadrant of the abdomen. Her fever increased markedly. The girl was admitted to the hospital, where, due to high leukocytosis, acute appendicitis (of the cecum) was suspected. An appendectomy was performed, but the appendix was found to be unchanged. Endoscopy revealed severe colonic inflammation (edema) and enlarged mesenteric lymph nodes. The child was treated with trimethoprim–sulfamethoxazole (TMP-SMX), and the symptoms subsided.

c) In the father, as in the boy, diarrhea and abdominal pain initially appeared and resolved by the fourth day. However, three weeks later, the man developed painful swelling of multiple joints and tender, red nodules on his legs. He received TMP-SMX, and his symptoms gradually resolved, but they recurred several months later. On that occasion, the symptoms spontaneously resolved after two weeks and did not recur.

Questions:

1. Which gastrointestinal pathogen can cause such varied clinical courses of infection depending on the age of the infected individual? ……
2. How did the infection occur? ……
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3. How can this infection be diagnosed? Is bacteriological testing necessary?
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4. Which antibiotics are effective in infections caused by this pathogen?
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5. Is antibiotic therapy necessary, given that symptoms in the youngest child resolved without treatment?
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6. Why did only the father and his children, but not the grandparents, develop the infection?
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7. Can such infections be prevented? Is there a vaccine?
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