Chapter 16. Gram-negative Bacilli Infections-I

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ENTEROBACTERALES

Enterobacterales include the commensal bacteria in the human intestine called coliform bacilli. They have the following general properties:

- They are gram-negative bacilli
- Aerobes and facultative anaerobes
- Nonfastidious, can grow in basal media like nutrient agar
- Ferment glucose and reduce nitrate

- All are catalase-positive, but oxidase test negative.

Based on the fermentation of lactose, Enterobacterales can be classified into:

- **Lactose fermenters (LF)**: Ferment lactose, and produce pink colonies on MacConkey agar; e.g. *Escherichia, Klebsiella, Enterobacter,* and *Citrobacter*
- **Non-lactose fermenters (NLF)**: Do not ferment lactose, produce pale colonies on MacConkey agar; e.g. *Salmonella*, *Shigella,* Proteeae *(Proteus, Morganella, Providencia),* and *Yersinia.*

Escherichia coli

Escherichia coli is the most common pathogen encountered clinically. It is also the most common aerobe to be harbored in the gut of humans.

Clinical Manifestations

Various strains of *E. coli* have been associated with various manifestations.

UTI by UPEC

Urinary tract infection (UTI) is caused by a strain of *E. coli* known as uropathogenic *E. coli* (UPEC), which is the most common cause (70–75%) of UTI. Infection to the bladder is usually spread by ascending route through the urethra, from the perineal flora.

Diarrhea (Diarrheagenic *E. coli)*

Diarrhea is caused by a strain of *E. coli* known as diarrheagenic *E. coli,* which further comprises six pathotypes.

1. **Enteropathogenic E. coli (EPEC):** It causes infantile diarrhea. It is nontoxigenic and noninvasive
2. **Enterotoxigenic E. coli (ETEC):** It causes traveler's diarrhea. Pathogenesis is mediated by producing toxins such as:
    - Heat labile toxin (LT): Acts by increasing cyclic AMP (similar to cholera toxin)
    - Heat stable toxin (ST): Acts by increasing cyclic GMP.
3. **Enteroinvasive E. coli (EIEC):** It is not toxigenic, but invasive and causes bloody diarrhea (i.e. dysentery)
4. **Enterohemorrhagic E. coli (EHEC):** The most common serotype associated with EHEC is O157:H7
    - Its pathogenesis is mediated by a toxin called verocytotoxin (or Shiga-like toxin)
    - It also causes dysentery, similar to EIEC
    - Verocytotoxin damages the endothelial cells causing capillary microangiopathy which may lead to complications such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis.
5. **Enteroaggregative E. coli (EAEC):** It causes persistent and acute diarrhea
6. **Diffusely adherent E. coli (DAEC):** It causes diarrhea in children aged 2–6 years.

Other Infections

Apart from UTI and diarrhea, *E. coli* can cause several pyogenic infections such as:

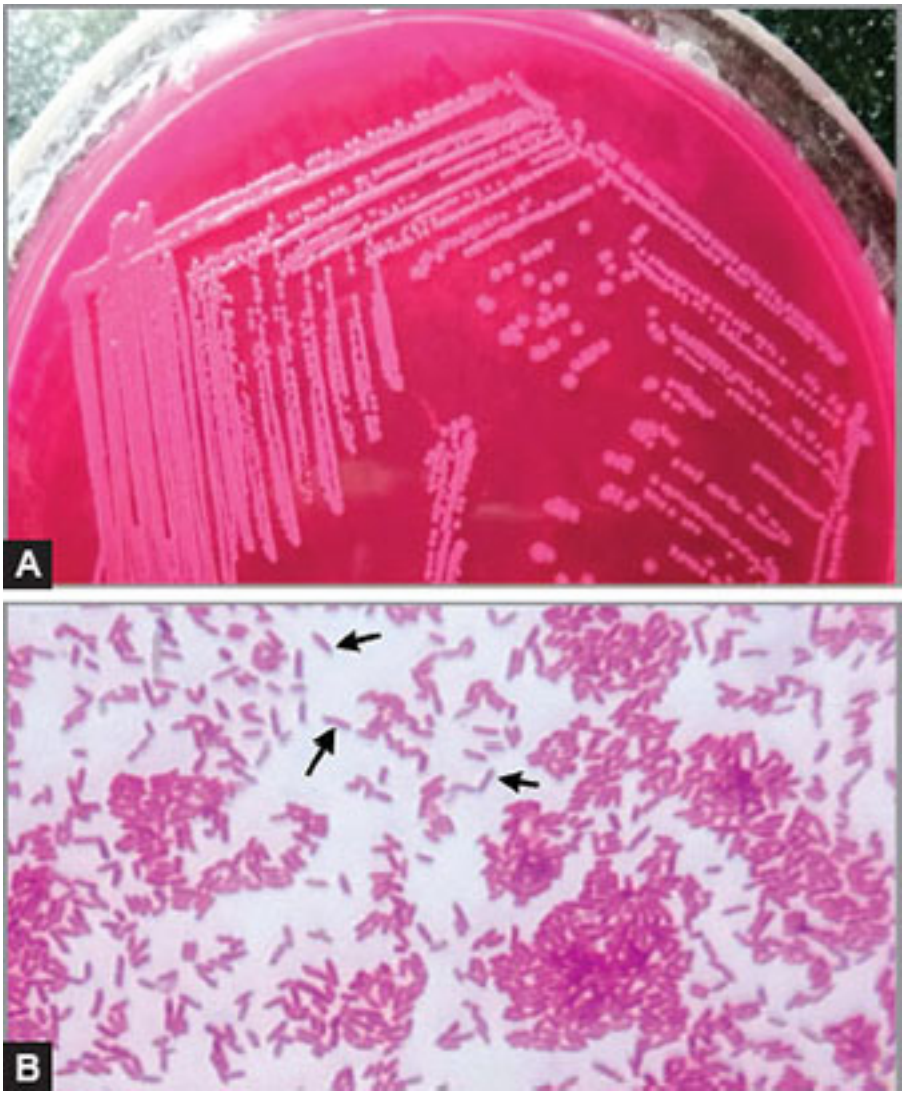
- **Abdominal infections:** Bacterial peritonitis (primary or secondary), visceral abscesses, such as hepatic abscess
- Pneumonia (especially in hospitalized patients—ventilator-associated pneumonia)
- Bloodstream infection (especially in hospitalized patients)
- Meningitis (especially neonatal meningitis)
- Wound and soft tissue infections such as cellulitis, infection of ulcers and wounds, especially in patient with diabetic foot.

Laboratory Diagnosis

Sample collection depends on the site of infection—urine, stool, pus, wound swab, blood, CSF, etc.

- **Direct smear microscopy:** Shows gram-negative bacilli, and pus cells
- **Culture:** Incubation at 37°C for 24h reveals the following growth:
  - Blood agar: Gray, moist colonies
  - MacConkey agar: Flat, pink LF colonies *(Fig. 16.1A)*
  - **Culture smear and motility:** Motile gram-negative bacilli *(Fig. 16.1B)*.
- **Biochemical identification:** Various biochemical tests which help in the identification of *E. coli* are:
  - Catalase positive and oxidase negative

Figs. 16.1A and B. *A.* Flat pink lactose fermenting colonies of *E. coli* on MacConkey agar; *B.* Slender gram-negative bacilli (arrows showing).



Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry *(with permission)*.

- ICUT tests: Indole, Citrate, Urease, Triple sugar iron (TSI) test are useful for identification.

- **Automated ID systems** such as VITEK and MALDI-TOF can be performed for rapid and accurate identification of *E. coli*

- **Antimicrobial susceptibility testing** can be performed by disk diffusion method (on Mueller-Hinton agar) or MIC-based method (VITEK).

TREATMENT

E. coli and Klebsiella

Treatment is essentially based upon an antimicrobial susceptibility test report. The majority of isolates in hospitals are multi-drug resistant (MDR) and require treatment with any of the following higher antimicrobials if found susceptible:

- Carbapenems such as meropenem
- #-lactam/#-lactamase inhibitor combinations (BL/BLIs) such as piperacillin-tazobactam or cefoperazone-sulbactam
- Aminoglycosides such as amikacin
- Polymyxins such as colistin
- Others: Fosfomycin or tigecycline, etc.

Preventive Measures

Infection control measures (contact precaution) such as hand hygiene are crucial to limit the spread of infection by multi-drug resistant Enterobacterales (refer Chapter 38).

Klebsiella pneumoniae

Similar to *E. coli, Klebsiella pneumoniae* can cause UTI, lobar pneumonia, meningitis (in neonates), septicemia, pyogenic infections such as abscesses, and wound infections.

- **Laboratory diagnosis:** Similar to *E. coli, K. pneumoniae* is also a lactose fermenter
  - It differs from *E.coli* in being non-motile, capsulated, and produces mucoid colonies *(Fig. 16.2)*
  - Identification can be done by various conventional biochemical tests such as catalase, oxidase, indole, citrate, urease, TSI or by automated ID systems such as VITEK and MALDI-TOF.
- **Treatment** for *K. pneumoniae* is the same as discussed for *E. coli.*

Other Klebsiella species include:

- **Klebsiella granulomatis:** It causes granuloma inguinale, a type of genitoulcerative disease
- **K. rhinoscleromatis** and *K. ozaenae:* Produce infections of the nasal cavity, called rhinoscleroma and atrophic rhinitis respectively.

Enterobacter species

Enterobacter species are similar to *Klebsiella* in clinical manifestations and also in most of the biochemical reactions except for being motile. *E. aerogenes* and *E. cloacae* are the most commonly isolated species from the clinical specimens. Treatment of *Enterobacter* infections is same as discussed for *E. coli.*

Citrobacter species

Citrobacter species are environmental contaminants, but species such as. *C. freundii* and *C. koseri* can cause human infections.

- **Manifestations:** They occasionally cause urinary tract, gallbladder and middle ear infections and neonatal meningitis
- **Identification** is made either by automated identification systems such as MALDI-TOF or VITEK; or by conventional biochemical tests

- **Treatment:** Most *Citrobacter* isolates are MDR, and the guideline for treatment is the same as that used for *E. coli*.

Fig. 16.2. *Klebsiella pneumoniae*; on MacConkey agar showing mucoid pink-colored lactose-fermenting colonies.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry *(with permission)*.

Salmonella

Salmonellae are broadly classified into two groups, based on the clinical disease produced:

1. **Typhoidal Salmonella:** It includes serotypes *S.* Typhi and *S.* Paratyphi. They are restricted to human hosts, in whom they cause enteric fever.
2. **Non-typhoidal salmonellae or NTS:** The remaining serotypes can colonize the intestine of a broad range of animals, including mammals, reptiles, birds, and insects. They also infect humans causing food-borne gastroenteritis and septicemia.

Enteric Fever

Enteric fever is a potentially fatal multisystem illness caused by *Salmonella* Typhi (typhoid fever) and, *S.* Paratyphi A, B and C (paratyphoid fever).

Pathogenesis

Salmonellae are transmitted by oral route, through ingestion of contaminated food or water.

- **The infective dose of** *Salmonella* is higher than that of *Shigella*. Minimum 10^3–10^6 bacilli are needed to initiate the infection

- **Risk factors** that promote transmission include the conditions that decrease gastric acidity and intestinal integrity
- **Primary bacteremia:** The bacilli enter through a specialized epithelial cell lining the intestinal mucosa—called M cells. Following this, they are internalized by macrophages and are carried to the bloodstream
- **Spread:** Then, the bacilli disseminate throughout the body such liver, spleen, lymph nodes and bone marrow, etc. where further multiplication takes place and then seeded back into the bloodstream **(secondary bacteremia)**, which leads to the onset of clinical disease.

Clinical Manifestations of Enteric Fever

The incubation period is about 10–14 days. Enteric fever is named after the mode of transmission (enteric route) of its causative agent. However, the manifestations seen are largely extraintestinal.

- **Fever (step ladder pattern of remittent fever):** Fever rises gradually to a higher level with every spike; then falls, but does not touch normal
- **Other symptoms:** Headache, chills, cough, sweating, myalgia, and arthralgia
- **Rashes (called rose spots):** Faint, salmon-colored, blanching, maculopapular rash on the trunk and chest seen in 30% of patients at the end of the first week
- **Early intestinal manifestations** such as abdominal pain, nausea, vomiting, constipation or diarrhea, and anorexia
- **Important signs** include hepatosplenomegaly, epistaxis, and relative bradycardia
- **Complications:** Gastrointestinal bleeding and intestinal perforation can occur mostly in the third and fourth weeks of illness
- **Neurologic manifestations** occur rarely which include meningitis, and neuropsychiatric symptoms such as delirium, etc.

Laboratory Diagnosis

(A) Blood Culture (First week of illness)

In the first week of illness, a blood culture is recommended.

- **Conventional blood culture** on media such as brain heart infusion (BHI) broth (monophasic media) or BHI broth/ agar (biphasic media)
- **Automated blood culture systems**—such as BACTEC or BacT/ ALERT
- **Blood culture positivity** is >90% in the first week and thereafter it gradually declines
- If blood culture is found negative, bone marrow culture or culture from duodenal aspirate may be performed in the first week of illness.

(B) Stool/urine Culture (in 3–4 weeks of illness)

Stool or urine culture is indicated in 3-4 weeks of illness, and also for detection of carriers:

- For *stool culture* the following media are used:
  - Enrichment broth such as Selenite F broth, tetrathionate broth, and gram-negative broth
  - Low selective medium such as MacConkey agar: Produces translucent NLF colonies
  - Highly selective media: DCA (deoxycholate citrate agar), XLD agar (xylose lysine deoxycholate), and Wilson Blair's Bismuth sulphite medium are used.

- A *urine culture* can be performed on media such as MacConkey agar.

(C) Identification

Salmonellae are motile, gram-negative bacilli.

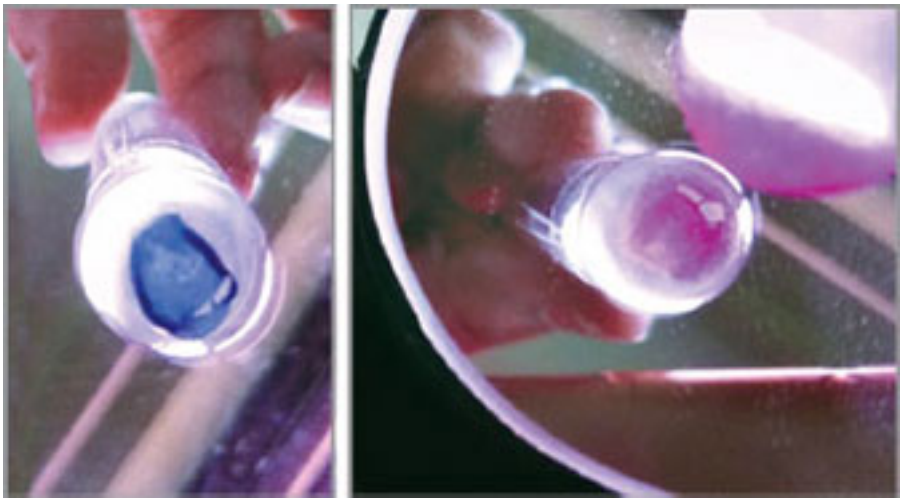
- Identification from the colonies grown in culture is made either by automated ID system such as VITEK or by conventional biochemical tests such as—catalase, oxidase, indole, citrate, urease and TSI
- **A slide agglutination test** is performed to confirm the serotype.

(D) Widal test (Serum antibody detection)

Widal test is indicated in 2–3 weeks of illness. It is a tube agglutination test, that detects antibodies in the patient's serum against antigens of *Salmonella* Typhi and *S.* Paratyphi.

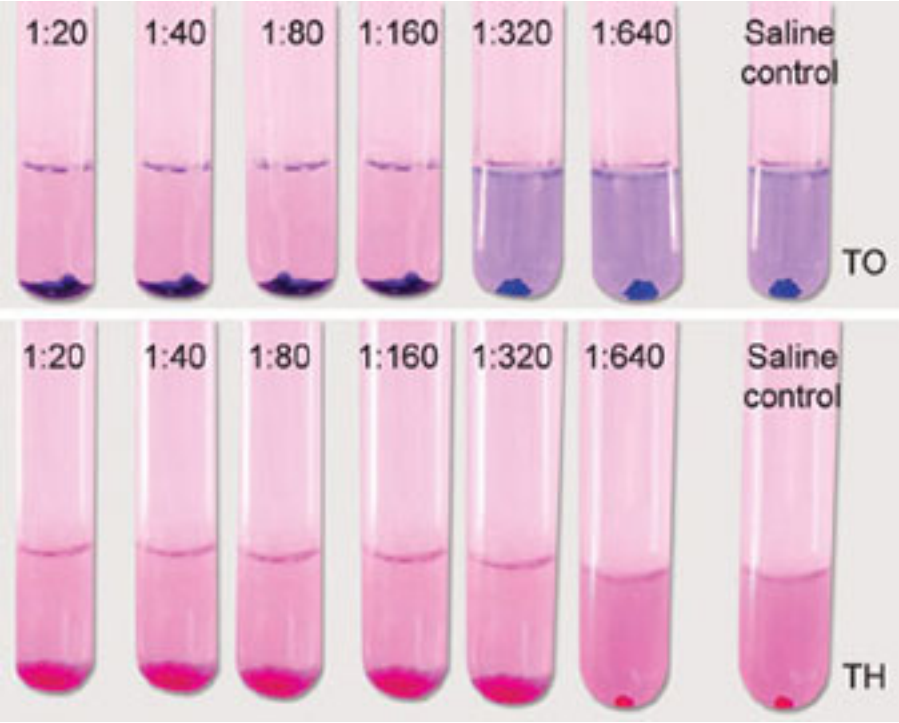
- **Antigens:** In the Widal test, four different antigens are used such as:
  - O antigen of *S*. Typhi (TO): It is cross-reactive to O antigens of *S*. Paratyphi A and B. Therefore, TO antigen can detect O antibody of *S*. Typhi, as well as *S*. Paratyphi A and B
  - H antigen of *S*. Typhi (TH)
  - H antigen of *S*. Paratyphi A (AH)
  - H antigen of *S*. Paratyphi B (BH)
- **Procedure:** Serial dilutions of patient serum are mixed with four different *Salmonella* antigens (TO, TH, AH, and BH) and the tubes are incubated in the water bath at 37°C for 24 hours
- **Result:** The result is read using a concave mirror. If corresponding antibodies are present, then an agglutination reaction will occur leading to matt formation. The absence of antibodies would lead to button formation *(Fig. 16.3)*
  - *O antibodies:* Produce granular chalky clumps when reacts with O Ag
  - *H antibodies:* Produce cottony woolly clumps when react with H Ag.
- **Significant titer:** H antibody titer of >1:200 is considered significant, whereas significant titer for O antibody is taken as >1:100. Low titers may be produced in cross-reacting infections and therefore should be ignored *(Fig. 16.4)*

Fig. 16.3. O and H agglutination in Widal test (reading taken in a mirror).



Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry *(with permission)*.

Fig. 16.4. Widal test showing titre of TO 1:160 and TH 1:320.



Abbreviation: TO and TH, antibody titre to *S.*Typhi O and H antigens in patient's serum.

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry *(with permission)*.

- **Interpretation:** The results are interpreted as below:
  - In *S.* Typhi infection: Antibodies to TO and TH antigens are raised
  - In *S.* Paratyphi A infection: Antibodies to TO and AH antibodies are raised
  - In *S.* Paratyphi B infection: Antibodies to TO and BH antibodies are raised.
- **False-negative:** The Widal test may produce a false-negative result in a very early stage (1st week) or due to prior antimicrobial therapy or due to prozone phenomena (antibody excess)
- **False-positive:** Widal test may produce a false-positive result in presence of cross-reacting infections (called anamnestic reactions).

(E) Other Tests

- **Antigen detection (serum and urine):** By ELISA
- **Molecular methods:** PCR detecting *flagellin* gene, *iro B* and *fli C* gene
- **Nonspecific findings:** For example, neutropenia
- **Antimicrobial susceptibility testing** can be performed by disk diffusion test or by MIC-based automated system (e.g. VITEK).

TREATMENT

Enteric fever

Third-generation cephalosporins such as ceftriaxone is the drug of choice for empirical treatment.

Alternative drugs are azithromycin, ciprofloxacin, chloramphenicol, ampicillin, and cotrimoxazole.

Vaccines for Typhoid Fever

There are two types of typhoid vaccines available currently.

1. **Vi antigen vaccine:** It is composed of purified Vi capsular polysaccharide antigen derived from *S.* Typhi strain Ty2
   - It is given as a single dose, by IM or subcutaneous route
   - The vaccine confers protection for 2 years; a booster is given every 2 years.
2. **Typhoral:** It contains live attenuated *S.* Typhi Ty21a strain
   - It is given orally as enteric-coated capsules
   - Four doses, given on alternate days
   - Revaccination is recommended every 5 years.

Shigella

Shigella is the causative agent of bacillary dysentery. It comprises four species—*S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei.*

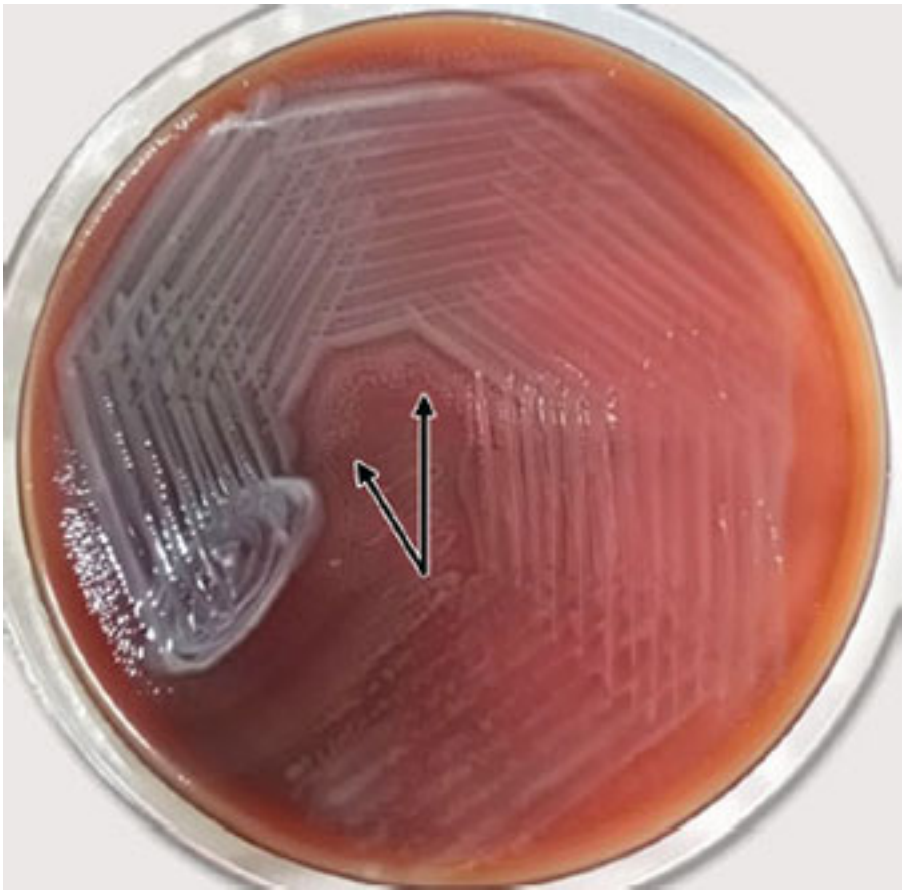
- **Transmission** of infection occurs by ingestion through contaminated fingers (most common), food, water, or rarely flies. Risk factors include overcrowding, poor hygiene, and children, etc.
- **Minimum infective dose:** As low as 10–100 bacilli are capable of initiating the disease, probably because of their ability to survive in gastric acidity
- **Pathogenesis** is due to the expression of various toxins such as—*Shigella* enterotoxin (by *S. flexneri),* Shiga toxin (by *S. dysenteriae)* endotoxin (by all species)
- **Clinical features:** Bacillary dysentery is characterized by the passage of loose stool mixed with blood and mucus
  - Shiga toxin *(S. dysenteriae)* is similar to verocytotoxin (of EHEC) and is associated with complications such as hemolytic uremic syndrome and hemorrhagic colitis
  - Rarely, may be associated with intestinal complications such as toxic megacolon, perforations, and rectal prolapse.
- **Laboratory diagnosis** includes isolation of organism from diarrheic stool specimen using enrichment medium such as selenite F medium and selective media such as DCA (deoxycholate citrate agar) or XLD (xylose lysine deoxycholate) agar; followed by identification by using appropriate biochemical reactions or automated ID method (like VITEK)
- **Antimicrobial susceptibility testing** can be performed by disk diffusion test or VITEK.
- **Treatment** of shigellosis includes fluid replacement and antimicrobials such as ciprofloxacin.

Tribe Proteeae

Tribe Proteeae comprises three genera: *Proteus, Morganella,* and *Providencia.*

- Although they are saprophytes and commensals; they can also cause opportunistic infections such as urinary tract infections, wound and soft tissue infections, septicemia and nosocomial outbreaks.

Fig. 16.5. *Proteus* on blood agar, showing swarming growth (arrows showing).



Source: Department of Microbiology, JIPMER, Puducherry *(with permission).*

Proteus is also involved in the pathogenesis of renal stones (struvite/phosphate stones)

- **Laboratory diagnosis:** *Proteus* produces characteristic swarming growth on blood agar *(Fig. 16.5)*. Identification of various members are made based on conventional biochemical tests or automated ID methods such as MALDI-TOF and VITEK. Antimicrobial susceptibility testing can be performed by disk diffusion test or VITEK

- **Treatment** is the same as discussed for *E. coli*, except that Tribe Proteeae are intrinsically resistant to certain antimicrobial agents (e.g. colistin, tigecycline, etc.) which should be avoided in the treatment

- The somatic antigen of certain non-motile *Proteus* strains (called X strains) can be used to detect cross-reacting heterophile antibodies in sera of patients suffering from rickettsial infections (Weil-Felix reaction).

Serratia

Serratia marcescens is usually a saprophyte in the environment, and typically produces a red non-diffusible pigment called prodigiosin. However, the hospital strains of *S. marcescens* are often non-pigmented and multiple drug-resistant and are associated with various nosocomial infections.

Yersinia species

Yersinia pestis (Plague)

It is the causative agent of plague, a fulminant systemic zoonosis; transmitted from rodents by the arthropod vector, the rat flea.

- **Epidemiology:** Plague was one of the greatest killer known to mankind; caused several pandemics in the ancient days producing millions of deaths. In India, the Surat epidemic (in 1994) has witnessed more than 6,000 suspected plague cases with 60 deaths
- **Clinical forms:** Human plague occurs in three clinical forms—(1) bubonic plague (most common form, characterized by enlarged and tender regional lymph nodes), (2) pneumonic plague, and (3) septicemic plague
- **Laboratory diagnosis:** Depending upon the type of plague, the specimens collected are: pus or fluid aspirated from buboes, sputum and blood
- **Direct microscopy:** Reveals gram- negative oval coccobacilli and pus cells
    - **Wayson staining** demonstrates *bipolar* or *safety pin* appearance of the bacilli
    - **Culture media** used are: Blood agar (non-hemolytic colonies) and MacConkey agar (NLF colonies)
    - **Identification** from colonies is either by automated identification systems (e.g. MALDI-TOF) or by conventional biochemical tests.
- **Treatment:** Streptomycin or gentamicin is recommended for treatment.

Yersiniosis

Infections due to other *Yersinia* species such as *Y. enterocolitica* or *Y. pseudotuberculosis* are called yersiniosis. They are enteropathogenic and cause gastroenteritis, terminal ileitis, and mesenteric lymphadenitis.

VIBRIO CHOLERAE

Vibrio cholerae is the causative agent of an acute diarrheal disease called cholera. It differs from Enterobacterales being oxidase-positive.

Typing

Typing of *Vibrio cholerae* can be done as follows:

- **Serogroups:** Based on the somatic O antigen, *V. cholerae* can be typed into several serogroups (>200). Out of which, serogroups O1 is the most common group to cause cholera, followed by serogroups O139
- **Biotypes:** Serogroup O1 can be typed based on biochemical reactions into two biotypes—classical and El Tor
    - Classical biotype is more virulent whereas El Tor biotype is more resistant to environmental stresses

  - Therefore classical biotype produces more severe illness, whereas El Tor biotype produces milder cases but more number of carriers.

- **Serotypes:** Serogroup O1 can be typed based on minor differences in O antigen into three serotypes—Ogawa, Inaba, Hikojima.

Epidemiology

The world has witnessed several cholera pandemics in the past; resulting in several thousands of deaths.

- **Seven pandemics** have been reported till date—first six were due to classical biotype and the seven one was due to El Tor biotype

- **Currently**, cholera occurs as sporadic and limited outbreaks. Majority of cases are due to El Tor, but cases due to classical biotype still occur in small proportion.

Pathogenesis

Pathogenesis of *V. cholerae* is due to a potent enterotoxin, called cholera toxin and a pilus (TCP).

- **Toxin-coregulated pilus (TCP):** It helps in the adhesion of the bacilli to the intestinal epithelium

- **Cholera toxin**: It is similar to the heat-labile toxin of *E. coli*, and has two fragments A and B

  - Fragment B binds to GM1 ganglioside receptors on the intestinal epithelium

  - Fragment A is the active unit, acts by increasing cyclic AMP

  - Cyclic AMP inhibits the absorption sodium and activates the secretion chloride, which lead to the accumulation of sodium chloride and water in the intestinal lumen and finally results in watery diarrhea.

Clinical Manifestations

Cholera manifests as painless watery diarrhea, described as a *rice-water stool*.

- **Mild fluid loss** may lead to features such as weakness, postural hypotension, tachycardia, and decreased skin turgor

- **Severe dehydration** can result in renal failure and fluid loss leading to—oliguria, weak or absent pulses, sunken eyes, wrinkled (“washerwoman”) skin and even coma.

Laboratory Diagnosis

Useful specimens are watery stool (for cases) or rectal swabs (for carriers).

- **Transport media:** Specimens should be sent in appropriate transport media such as VR medium (Venkatraman–Ramakrishnan), and Cary-Blair medium

- **Direct microscopy** of the stool specimen reveals:

  - Gram stain: Gram-negative rods, short curved comma-shaped (fish in stream appearance)

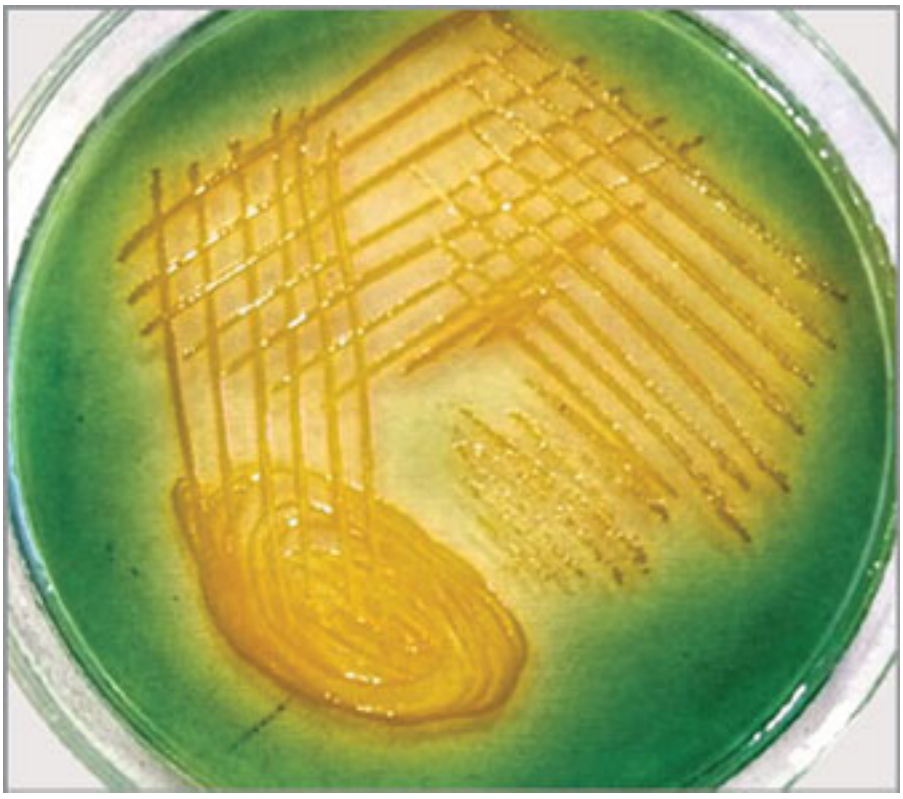
  - Hanging drop method: Demonstrates *darting motility*—extremely active motility with rapid changing direction.

- **Culture:** Various culture media used for *V. cholerae* are:

- **Enrichment broth:** Alkaline peptone water, Monsur's taurocholate tellurite peptone water
- **Selective media**: (i) Bile salt agar, (ii) Monsur's gelatin taurocholate tellurite (GTT) agar, and (iii) thiosulfate citrate bile salts sucrose (TCBS) agar
- **TCBS agar:** It is the most common selective media used for *V. cholerae*, which produces typical yellow colonies *(Fig. 16.6)*
- **MacConkey agar:** *V. cholerae* produces translucent NLF colonies.

- **Culture smear and motility testing**—reveal short curved gram-negative bacilli and darting motility
- **Identification:** *V. cholerae* produces hemodigestion on blood agar and gives positive string test. Identification of *V. cholerae* from the colonies can be performed by following tests:
  - Conventional biochemical tests such as oxidase, catalase, indole, citrate, urease and TSI test
  - Automated methods such as MALDI-TOF and VITEK.
- **Typing:** After being identified as *V. cholerae*, it is further subjected to various typing methods

Fig. 16.6. Thiosulfate citrate bile salts sucrose (TCBS) agar with yellow-colored colonies of *Vibrio cholerae*.



Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry *(with permission)*.

- *Biotyping:* To differentiate classical and El Tor
- *Serogrouping:* To differentiate O1 and O139

- *Serotyping:* To differentiate Ogawa, Inaba, and Hikojima serotypes of serogroup O1.

- **Antigen detection** can be done by tests such as cholera dipstick assay
- **Molecular method:** Multiplex PCR can be used to detect common diarrheal pathogens
- **Antimicrobial susceptibility testing** can be performed by disk diffusion test or by automated methods (e.g. VITEK).

TREATMENT

Cholera

Fluid replacement is the mainstay of treatment of cholera. Antibiotics such as macrolides (azithromycin) or doxycycline can be given to severely dehydrated patients.

Cholera Vaccine

Oral cholera vaccines (OCV) are currently in use for the prevention of cholera. They usually give short-term protection (6 months or so). Two types of OCVs are available.

1. **Killed whole-cell vaccine,** e.g. include whole-cell (WC) vaccine (e.g. Shanchol) and whole-cell recombinant B subunit vaccine (e.g. Dukoral)
2. **Oral live attenuated vaccines,** e.g. CVD 103-HgR vaccine.

Injectable killed vaccines which were used before are no longer used.

Halophilic Vibrio

The *Vibrio* species other than *V. cholerae* that grow in higher salt concentrations are called *halophilic Vibrios*; examples include *V. parahaemolyticus, V. alginolyticus* and *V. vulnificus.* They cause intestinal and extraintestinal manifestations.

EXPECTED QUESTIONS

1. I. Write essay on:

1. Discuss the pathogenesis, clinical manifestations, laboratory diagnosis, and treatment of enteric fever.
2. Discuss the epidemiology, clinical manifestations, laboratory diagnosis and treatment of cholera.

2. II. Write short notes on:

1. Diarrheagenic *E. coli.*
2. *Klebsiella pneumoniae* infections.
3. Shigellosis.
4. Plague.

3. III. Multiple Choice Questions (MCQs):

1. **TCBS agar is used for_____?**

a. *Vibrio cholerae*

b. *Salmonella*

c. *Shigella*

d. Mycobacteria

2. **Which of the following exhibits darting motility?**

a. *Salmonella*

b. *Shigella*

c. *Vibrio cholerae*

d. *E. coli*

3. **In first week of illness, enteric fever is diagnosed by_____?**

a. Widal test

b. Blood culture

c. Stool culture

d. Urine culture

Answers

1. a	**2.** c	**3.** b