ENTEROBACTERIACEAE

COMPLETE PPT-BASED REVIEWER

Compiled from the entire uploaded Enterobacteriaceae PowerPoint

Coverage	All substantive content from the 1,218-line PPT extraction
Includes	General characteristics • classification • MacConkey/EMB/SS/TSI • E. coli • EPEC/ETEC/EIEC/EHEC/EAEC/DAEC • Klebsiella • Enterobacter • Serratia • Hafnia • Proteus/Morganella/Providencia • Citrobacter • Salmonella • Shigella • Edwardsiella • Yersinia • biochemical test principles
Important	This reviewer is organized and condensed for studying, but it preserves the PPT's terminology and points rather than silently replacing them with outside textbook information.

- Study tip: memorize the bolded organism/test associations first, then use the detailed sections for understanding.
- The biochemical-test section is included because it is a major part of the uploaded PPT and was underrepresented in the earlier reviewer.

1. GENERAL CHARACTERISTICS OF ENTEROBACTERIACEAE

- Largest and most medically important family of gram-negative bacilli (GNB).
- More than 90% of medically important isolates belong to just a few genera.
- Found worldwide and part of the normal microbiota of all animals.
- Common cause of nosocomial infections.
- All members ferment glucose, reduce nitrates to nitrites, and are oxidase negative; the PPT notes Plesiomonas as an oxidase-positive exception and says phylogenetic studies place it close to Proteus.
- Antigens include O/somatic antigen, H/flagellar antigen, and K/capsular antigen.
- Commonly present in the large intestine.
- Facultatively anaerobic; non-sporing; non-acid-fast; gram-negative bacilli.
- Complex family: some are nonpathogenic, a few are highly pathogenic, and commensals can become pathogenic (for example, UTI after catheterization).
- Motile except Shigella and Klebsiella.
- Non-capsulated except Klebsiella.
- Non-fastidious.
- Grow on bile-containing media such as MacConkey agar.

2. SELECTIVE/DIFFERENTIAL MEDIA AND LACTOSE FERMENTATION

- Important media listed in the PPT: MacConkey agar, Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar, and Triple Sugar Iron (TSI) agar.
- MacConkey agar is selective and differential for Enterobacteriaceae.
- MacConkey components listed: bile salts, crystal violet, lactose, and neutral red.
- Bile salts and crystal violet inhibit gram-positive bacteria and provide selectivity.
- Lactose fermentation produces acid; neutral red is the pH indicator.
- Lactose fermenters (LF) appear pink; non-lactose fermenters (NLF) appear colorless.
- Method given: streak inoculate MacConkey agar and incubate at 37°C for 24 hours.
- Example LF: E. coli → pink colonies.
- Example NLF: Shigella → colorless colonies.
- Basic classification in the PPT: lactose fermenters/coliforms (E. coli, Escherichia, Klebsiella); late lactose fermenter (Shigella sonnei); non-lactose fermenters (Salmonella, Shigella).
- The PPT notes Shigella sonnei is pronounced 'son-ya' and Shigella flexneri 'flex-ne-ra'.
- LF is also called 'para colons' in the PPT.

3. TAXONOMICAL CLASSIFICATION

- Tribe I: Escherichia → Escherichia and Shigella.
- Tribe II: Edwardsiella → Edwardsiella.
- Tribe III: Salmonelleae → Salmonella.
- Tribe V: Klebsiella → Klebsiella, Enterobacter, Hafnia, Serratia, Pantoea, Cronobacter.
- Tribe VI: Proteae → Proteus, Morganella, Providencia.
- Tribe VII: Yersinieae → Yersinia.

4. HIGHLY PATHOGENIC ENTEROBACTERIACEAE

- Salmonella.
- Shigella.
- Escherichia coli pathotypes listed: EPEC, EIEC, EHEC, ETEC, EAEC/DEAC.
- Shigella colonies on MacConkey are lactose-negative and therefore colorless.

5. ESCHERICHIA COLI — MORPHOLOGY AND BASIC BIOCHEMISTRY

- Gram-negative straight rods, approximately 1–3 × 0.4–0.7 µm.
- Appear singly or in pairs.
- Motile by peritrichous flagella; very few strains are nonmotile.
- Non-spore-forming and non-acid-fast.
- Aerobic/facultatively anaerobic.
- Grows between 10–40°C; optimum 37°C.
- Grows on simple media.
- Produces large grayish, thick white, moist, smooth, opaque colonies; may contain capsule.
- On MacConkey: bright pink lactose-fermenting colonies.
- Many pathogenic strains are hemolytic on blood agar.
- Ferments glucose, mannitol, and maltose with gas.
- IMViC: ++–+.
- Urease negative.

6. E. COLI — ANTIGENS AND VIRULENCE FACTORS

- Two broad types of virulence factors in the PPT: surface antigens and toxins.
- O/somatic LPS antigen: heat-stable; has endotoxin activity; protects against phagocytosis and complement bactericidal effects.
- K/capsular antigen: heat-labile; protects against phagocytosis.
- H/flagellar antigen: heat-labile.
- Fimbriae are present in large numbers and promote virulence; especially important in UTI.
- PPT antigen counts: O = 170, K = 100, H = 75.
- E. coli produces exotoxins including hemolysins/cytolysins and enterotoxins.
- Hemolysins/cytolysins and enterotoxins are associated with diarrhea.
- Enterotoxins include heat-labile (LT) and heat-stable (ST) toxins.
- Verotoxins are Shigella-like toxins.

7. E. COLI ENTEROTOXIN MECHANISMS

- Enterotoxins cause movement of water and ions from tissues into the bowel, producing watery diarrhea.
- LT is heat-labile and binds specific gangliosides on small-intestinal epithelial cells.
- LT ADP-ribosylates Gs, stimulating adenylate cyclase and increasing cAMP.
- Increased cAMP alters sodium/chloride transporter activity, producing ion imbalance and movement of fluid into the bowel.
- The PPT describes LT as having an A/B arrangement: A is activated into A1/A2; A is the active component and B is the binding portion.
- A1 activates adenylate cyclase in enterocytes to form cyclic AMP, increasing water/electrolyte outflow into the gut lumen.

- ST is described as A and B fragments and activates cyclic GMP (cGMP), causing fluid accumulation in the intestine.
- Both enterotoxins are described as five beta subunits for binding and one alpha subunit with toxic enzymatic activity.
- B confers specificity and binds GM1 ganglioside of intestinal mucosa, permitting entry of A.
- Verotoxin acts like Shigella dysentery toxin.
- VT1 is described as identical to Shiga toxin from S. dysenteriae type 1 and damaging Vero cells (African green monkey kidney cells).

8. E. COLI — CLINICAL INFECTIONS

- Neonatal meningitis and septicemia: leading cause of neonatal meningitis in the PPT; high mortality; usually associated with K1 capsular antigen.
- Gastroenteritis: ETEC, EIEC, EPEC, EAEC, and EHEC are listed.
- UTI: leading cause; can cause acute cystitis and pyelonephritis; mostly ascending UTI.
- Pyogenic infections.
- Intra-abdominal infections including peritonitis, abscess, and septicemia.
- Drug-resistant infections occur.
- UTI risk factors listed: female sex/short urethra, pregnancy, sexual intercourse ('honeymoon cystitis'), urethral obstruction, urinary stones, congenital malformations, neurologic disorders, catheterization, and cystoscopy.
- Cystitis is usually produced by fecal strains entering the urethra.

9. EPEC — ENTEROPATHOGENIC E. COLI

- Causes diarrheal disease in children.
- PPT associates EPEC with O26/O11 and describes verocytotoxin production.
- Causes infantile enteritis involving the upper intestine.
- Brush border of the intestine is lost/disrupted.
- Intimacin is identified as an EPEC adhesion factor.
- Frequent in summer months; poor hygiene predisposes; outbreaks occur in institutions.
- Identification described by serotyping O and B antigens; diagnosis may be difficult in sporadic cases.
- Routine culture is used for isolation.
- The PPT states EPEC fails to ferment sorbitol.
- Laboratory diagnosis: polyvalent and monovalent antisera for serogroups; HEp-2 adherence.

10. ETEC — ENTEROTOXIGENIC E. COLI

- Produces heat-stable and heat-labile toxins.
- Adheres to small-intestinal epithelium.
- Produces nausea, vomiting, and loose stool.
- Heat-labile toxin is described as cholera-toxin-like and causes fluid accumulation.
- Adhesive factors include fimbriae/colonization factor antigens (CFA) that bind specific intestinal receptors.
- Mortality concern in children under 5 years.
- Common and causes endemic disease in developing countries and all age groups.
- Disease ranges from mild watery diarrhea to fatal conditions.
- Important cause of traveler's diarrhea; water contamination is a predisposing factor.
- Treatment/prevention listed in the PPT: doxycycline, trimethoprim, norfloxacillin, fluoroquinolones; avoid contaminated food; use safe/protected water (prefer bottled); hot foods/drinks; boiled milk.

- Laboratory diagnosis: demonstration of LT and ST; tissue culture; ELISA; passive agglutination; rabbit ileal loop test.

11. EIEC — ENTEROINVASIVE E. COLI

- Resembles Shigella in many respects.
- Non-lactose fermenter and nonmotile in the PPT.
- Invades intestinal epithelium.
- Penetrates HeLa cells in tissue culture.
- May cause mild diarrhea to frank dysentery.
- Some strains are nonmotile.
- Sereny test positive in rabbit; ELISA is listed.

12. EHEC — ENTEROHEMORRHAGIC E. COLI / SHIGA-LIKE TOXIN PRODUCING

- Produces verocytotoxin/Shiga-like toxin.
- Disease can range from mild diarrhea to fatal hemorrhagic colitis and hemolytic uremic syndrome (HUS).
- Present in human and animal feces.
- O157 is highlighted in Japan and the USA; typical serotype listed as O157:H7.
- Associated foods/environmental exposures in the PPT include salads, vegetables, radish; proper cooking recommended.
- Primary toxin target appears to be vascular endothelial cells; renal capillary microangiopathy contributes to HUS.
- Disease may manifest as food poisoning due to fecal contamination from humans or animals.
- HUS: Shiga-like toxin enters circulation by binding Gb3 receptors; greatest receptor concentration is described in kidneys, especially in children.
- Laboratory diagnosis: culture, DNA detection methods, cytotoxic effects on Vero cells, and monovalent O157/H7 sera.

13. EAEC — ENTEROAGGREGATIVE E. COLI

- Appears in stacked-brick aggregation on HEp-2 cells.
- Produces persistent diarrhea.
- Produces a low-molecular-weight heat-stable enterotoxin.
- Laboratory diagnosis: stool culture and detection of enterotoxin.

14. DAEC — DIFFUSELY ADHERENT E. COLI

- Closely associated with cystitis in children and acute pyelonephritis in pregnant women.
- Also associated with chronic or recurring UTIs.
- Associated with pediatric diarrheal disease.

15. TREATMENT OF E. COLI-RELATED GI DISEASE

- Fluid replacement is emphasized.
- Antibiotics are not recommended unless systemic disease/HUS is involved according to the PPT.
- When indicated, drugs listed include sulfonamides, ampicillin, cephalosporins, aminoglycosides, and tetracyclines.

16. KLEBSIELLA, ENTEROBACTER, SERRATIA, HAFNIA

- Usually found in the intestinal tract; associated with pneumonia, wounds, and UTI.

- General characteristics: some nonmotile; Simmons citrate positive; H2S negative; phenylalanine deaminase negative; some weakly urease positive; MR negative and VP positive.

Klebsiella species

- Usually found in GI tract; four major species are highlighted, with K. pneumoniae most commonly isolated.
- Prominent polysaccharide capsule protects against phagocytosis and antibiotics and produces moist, mucoid colonies.
- Distinctive 'yeasty' odor.
- Frequent cause of nosocomial pneumonia.
- Seven species listed in the PPT: K. pneumoniae, K. ozaenae, K. rhinoscleromatis, K. oxytoca, K. planticola, K. terrigena, K. ornithinolytica.
- K. pneumoniae is most medically important; K. oxytoca and K. rhinoscleromatis also occur in clinical specimens.
- NF of GI tract but potential pathogen elsewhere.
- TSI A/A + gas; LIA K/K; urea +; citrate +; MR–/VP+; motility –; O and K antigens.
- Lactose positive; most are urease positive; nonmotile.
- Virulence factors: capsule, adhesins, iron-capturing ability.
- Clinical: pneumonia, especially in immunocompromised hosts; permanent lung damage is highlighted; major nosocomial cause of septicemia and meningitis.

Enterobacter species

- 12 species; E. cloacae and E. aerogenes are most common in the PPT.
- Isolated from wounds, urine, blood, and CSF.
- Colonies resemble Klebsiella.
- Motile.
- MR negative; VP positive.

Serratia species

- Seven species; S. marcescens is the only clinically important species in the PPT.
- Frequently found in nosocomial urinary or respiratory infections.
- Implicated in bacteremic outbreaks in nurseries, cardiac surgery, and burn units.
- Fairly resistant to antibiotics.
- Ferments lactose slowly.
- Produces characteristic pink pigment, especially when cultures are left at room temperature.

Hafnia species

- Hafnia alvei is the only species listed.
- Isolated from many anatomical sites and the environment; occasionally isolated from stools.
- Delayed citrate reaction is a major characteristic.

17. PROTEUS, MORGANELLA, PROVIDENCIA

- Part of normal GI flora in the PPT except Providencia.
- All are motile; Proteus shows swarming.
- Phenylalanine deaminase positive.
- Lysine deamination positive (LIA R/A).
- Urease positive for most; strongly positive for Proteus.

- TSI is variable; the PPT emphasizes knowing each reaction in the lab.
- Indole: only P. mirabilis is negative.
- Proteus, Morganella, and Providencia are opportunistic pathogens, deaminate phenylalanine, and are lactose negative.

Proteus

- P. mirabilis and P. vulgaris are recognized human pathogens.
- Isolated from urine, wounds, ear infections, and bacteremic infections.
- Both produce swarming colonies on nonselective media and have a distinctive 'burned chocolate' odor.
- Both are strongly urease positive and phenylalanine deaminase positive.

Morganella

- M. morganii is the only species listed.
- Documented cause of UTI and isolated from other anatomical sites.
- Urease positive; phenylalanine deaminase positive.

Providencia

- P. rettgeri is a urinary tract pathogen and has caused nosocomial outbreaks.
- P. stuartii can cause nosocomial outbreaks in burn units and has been isolated from urine.
- Both are phenylalanine deaminase positive.

18. CITROBACTER

- C. freundii is associated with nosocomial UTI, pneumonia, and intra-abdominal abscesses.
- Ferments lactose and hydrolyzes urea slowly.
- Resembles Salmonella.

19. SALMONELLA

- More than 2,400 serologic types based on O, H, and Vi antigens.
- Motile.
- Do not ferment lactose or sucrose.
- Produce H2S.
- Resistant to sodium deoxycholate, brilliant green, and sodium tetrathionate; these inhibit other enteric organisms.
- Positive lysine decarboxylase, negative indole and ONPG are characteristic.
- S. typhi is differentiated from other Salmonella in the PPT by negative citrate and ornithine decarboxylase.
- Salmonella are overt pathogens and may cause severe infections in patients with AIDS.
- Clinical syndromes: typhoid fever/enteric fever, bacteremia, enterocolitis.
- S. typhi is the major cause of typhoid fever and is found exclusively in humans.

Typhoid fever

- Large number of organisms must be ingested because they are susceptible to stomach acidity.
- Bacteria proliferate in Peyer's patches, which become infiltrated with inflammatory cells.
- Organisms travel through lymphatic and blood circulation and disseminate to gallbladder, liver, spleen, kidneys, and other sites.
- Incubation approximately 2 weeks; fever rises to a high plateau and may persist 4–8 weeks untreated.

- First week: constipation and then bloody diarrhea are described; weakness, stomach pain, headache, anorexia, hepatomegaly, and splenomegaly may occur.
- Necrotic liver lesions and inflammation of gallbladder, lungs, periosteum, and other organs may occur.
- Rose spots may appear on abdomen while bacteria are in blood.
- WBC count is normal or low.
- Blood cultures positive during first and second weeks; stool cultures positive from second week onward.
- Carrier state in 2–5% after resolution; gallbladder is major reservoir and is described as a good culture medium.
- Carriers shed bacteria in stool.
- Recovery provides some immunity; intestinal IgA may prevent attachment; serum antibodies to O and Vi are associated with resistance.

Salmonella bacteremia

- Focal lesions are most often seen after S. choleraesuis but can follow virtually any serotype.
- Elderly/debilitated persons, people with chronic disease, and children with enterocolitis are most susceptible.
- Blood cultures positive during high fever; diarrhea usually absent; organisms rarely isolated from stool.
- Incubation variable; abrupt onset; early invasion of blood; rapidly rising fever with spikes; lesions may develop in lungs, bones, and meninges.

Salmonella enterocolitis / gastroenteritis

- Most common manifestation of Salmonella infection.
- S. typhimurium and S. enteritidis are most commonly isolated.
- Diarrhea, fever, nausea, vomiting, and headache occur 8–48 hours after ingestion.
- Low-grade fever and intestinal inflammation are common.
- Usually resolves within 4–7 days.
- Stool cultures may remain positive for several weeks after recovery.
- Most patients do not require treatment except neonates; treatment may cause organisms to retreat to gallbladder and create a carrier state.
- Typhoid fever and bacteremia require appropriate antibiotics.

Salmonella treatment, vaccines, prevention

- Fluoroquinolone listed as empiric drug of choice for typhoid fever.
- Ampicillin, TMP-SXT, and third-generation cephalosporins may be effective.
- Multidrug resistance is a problem; the PPT associates this with antibiotic use as growth promoters in food animals.
- Chronic-carrier eradication may require long-term ampicillin with cholecystectomy.
- Vaccines are primarily used during outbreaks.
- Live attenuated rough-strain S. typhi vaccine: oral capsule, four doses with 2-day intervals; PPT states 5 years protection.
- Purified Vi polysaccharide vaccine: single injection; PPT states 2 years protection.
- Neither vaccine provides absolute immunity; disease may occur after a large enough inoculum.
- Prevention: proper sewage disposal, unpolluted water, proper handling of animal-origin foods, proper cooking, health control of public eating places, and identification/treatment of carriers.
- Recovered typhoid patients may shed S. typhi for a long time, exposing family members and close contacts.

20. SHIGELLA

- Four serogroups: A S. dysenteriae (12 serotypes); B S. flexneri (6); C S. boydii (23); D S. sonnei (1).

- Transmission is person-to-person via the 5 F's: friends, flies, fingers, feces, food; contaminated water also contributes.
- Naturally found only in intestinal tracts of humans and some non-human primates.
- Historically described as a disease of armies; now emphasized as a disease of institutions/crowded substandard living conditions.
- Gram-negative slender rods, often coccobacillary in early cultures.
- Nonmotile because they lack flagella.
- Facultative anaerobes.
- Do not ferment lactose.
- Ferment glucose and other carbohydrates with acid but no gas.
- BAP colonies are convex and transparent.
- Four serogroups can be differentiated by mannitol, lactose, and indole.
- Causes bacillary dysentery/shigellosis; S. dysenteriae causes the most severe disease.
- Disease involves LPS endotoxin and Shiga exotoxin from S. dysenteriae.
- Highly infectious; ingestion of fewer than 1,000 organisms can cause disease.
- Shiga toxin can act as enterotoxin and neurotoxin; PPT describes meningismus, coma, and death.
- After recovery, most individuals shed organisms for a short period; serum antibodies develop but are not protective against reinfection.
- Treatment listed: ciprofloxacin, ampicillin, TMP-SXT, tetracycline, chloramphenicol if necessary.
- Prevention: sanitary food/water, sewage disposal, disinfection of feces, fly control, isolation, and identification/treatment of subclinical cases and carriers.

21. EDWARDSIELLA TARDA

- Primarily found in freshwater fish; causes hemorrhagic septicemia in commercially important fish.
- Also isolated from turtles, lizards, snakes, alligators, crayfish, birds, mammals, lakes, rivers, and sewage.
- Motile gram-negative rods producing H_2S.
- Ferments glucose with gas; does not ferment lactose, sucrose, or most other sugars.
- Majority positive for indole, MR, lysine/ornithine decarboxylases.
- Negative for VP, citrate, urease, phenylalanine deaminase, gelatin, and arginine dihydrolase.
- Morphologically resembles Salmonella.
- Intrinsically resistant to colistin; a sensitive susceptibility result should prompt investigation for identification error.
- In humans, intestinal tract is the main localization; 80% of cases are associated with GI disease.
- Can cause fish-hook wound infections, osteomyelitis, peritonitis, myonecrosis, cholecystitis, and sepsis.
- May be isolated from stool, blood, urine, CSF, peritoneal fluid, and bile.
- PPT states it is almost always susceptible to antibiotics commonly used for gram-negative bacteria.

22. YERSINIA

- Facultatively anaerobic or microaerophilic.
- Short, ovoid-to-rod-shaped gram-negative bacilli.
- Nonmotile at 37°C; some motile below 30°C.
- PPT states they are not found in the intestinal tract of humans or animals.
- Bipolar 'safety-pin' appearance with Wayson, Wright, or Giemsa stain, but not on Gram stain.
- Y. pestis is the notorious plague-causing species.
- Y. enterocolitica and Y. pseudotuberculosis cause enterocolitis and mesenteric adenitis.

- Produce many virulence factors.
- Ferment mannitol but not lactose; PYR, MR, and catalase positive; citrate negative.
- Growth relatively slow on blood and MacConkey agar; best growth at 25–28°C.
- BAP colonies resemble fried eggs.
- Specimens listed: blood, aspirates/enlarged lymph nodes, sputum, CSF.
- Isolation/identification described as requiring BSL-3 facilities.
- Peripheral growth around the central colony contains capsular material that forms strings when scooped with a loop.
- Ferment glucose and mannitol; urease negative.

Yersinia pestis virulence factors

- LPS.
- Fraction I (FI): heat-labile envelope protein produced mainly at 37°C; described as the main antigen responsible for virulence and immunity.
- V-W antigen system.
- Exotoxin causing hemoconcentration and shock.
- Coagulase produced at 28°C.
- Bacteriocin that kills some bacteria.
- Serum antibody titer >16 is presumptive evidence; a two-fold rise in acute/convalescent serum confirms diagnosis.
- PPT states Y. pestis LPS is 50–100 times more potent than enteric bacterial LPS.
- V antigen is cell-bound protein; W antigen is excreted lipoprotein.
- For lethal infection, Y. pestis must produce at least the first four factors listed.

Bubonic plague

- Acquired after infected-flea bite, direct contact with infected animals (rat/ground squirrel), or inhalation of contaminated aerosols.
- Robust capsule production at 37°C permits organisms to escape destruction and travel via lymphatics to lymph nodes.
- Lymph nodes swell up to ~4 cm and form buboes.
- Organisms may be phagocytized by neutrophils/macrophages but can multiply intracellularly and extracellularly.
- Bubonic plague: painful buboes, high fever, delirium, vomiting, diarrhea, edema, DIC.
- Untreated fatality rate described as 50% within 3–5 days.

Pneumonic plague

- Occurs when buboes burst and pneumonic disease ensues, releasing organisms into blood or through contaminated aerosols.
- Lungs fill with fluid; sputum is bloody/frothy; severe breathing difficulty.
- Drug of choice listed: streptomycin; chloramphenicol and tetracycline also effective.
- Isolation of bubonic or pneumonic plague patients is mandatory.
- Bubonic plague has high incidence of pulmonary involvement and aerosol spread.
- PPT calls Y. pestis the 'ultimate pathogen' because of high virulence and rapid killing.

Y. enterocolitica and Y. pseudotuberculosis

- Found in feces and lymph nodes of healthy and sick animals.
- Transmission mainly by ingestion of food/water contaminated with animal feces; person-to-person transmission is rare.
- Fever, abdominal pain, and bloody diarrhea may appear after 5–10 days.
- Multiplication in gut, especially ileum, causes inflammation, ulceration, and blood in stool.

- CIN (Cefsulodin-Irgasan-Novobiocin) is used; fecal specimens are inoculated and incubated 48 h.
- Aeromonas may grow on CIN, but most other bacteria will not.
- Cold enrichment: fecal sample/rectal swab in buffered saline pH 7.6 at 4°C for 2–4 weeks.
- Specimens: stool and rectal swabs; BAP, MacConkey, HE agar, and XLD may be used.
- CIN is specifically designed for Y. enterocolitica; colonies look like a target with a red center and clear edge.
- Species rapidly produce urease.
- Motile at room temperature (25°C) but not at 37°C.
- Y. enterocolitica has >50 serotypes; Y. pseudotuberculosis has at least 6.
- Y. enterocolitica is ornithine decarboxylase positive and ferments sucrose; Y. pseudotuberculosis is negative for both.

23. MEDIA AND TESTS USED FOR IDENTIFICATION

- The PPT introduces culture media and biochemical tests for isolation, selection, differentiation, and identification of Enterobacteriaceae.

Triple Sugar Iron (TSI) agar

- Phenol red is the indicator.
- Lactose and sucrose are present at 10× the concentration of glucose.
- Glucose is metabolized first; anaerobic fermentation produces an acidic butt.
- This is followed by fermentation of sucrose or lactose when present, producing an acid slant and butt.
- If sucrose/lactose is not used, peptones/proteins on the slant are used, making the slant alkaline.

Nitrate reduction

- Determines ability of organism to reduce nitrate.
- Nitrate reduction to nitrite is detected by sulfanilic acid and alpha-naphthylamine.
- Sulfanilic acid + nitrite → diazonium salt; diazonium salt couples with alpha-naphthylamine to produce a red, water-soluble azo dye.
- PPT says the test is read for gas, nitrate, and nitrite.
- Zinc dust is added to ensure nitrite has not been oxidized to nitrate.
- If there is no color change, zinc is added to confirm a negative result.
- After zinc: red color = true negative (organism did not reduce nitrate to nitrite).
- After zinc: no red = positive (organism reduced nitrate beyond nitrite to nitrogen gas); gas may be present in Durham tube.
- NF in the PPT = reduction of nitrate to nitrogen gas.

Oxidase

- Detects bacterial cytochrome oxidase by oxidation of 1% tetramethyl-p-phenylenediamine dihydrochloride to indophenol.
- Indophenol is a dark-purple end product.

Indole

- Determines presence of tryptophanase.
- Tryptophanase breaks tryptophan into indole, pyruvic acid, and ammonia.
- Indole is detected using Kovac's reagent.
- The PPT describes indole + aldehyde (cinnamaldehyde) producing a colored compound.

Methyl Red (MR)

- Determines ability to produce and maintain stable acid end products from glucose fermentation.

- Glucose fermentation produces high amounts of lactic acid, reacting with methyl red to give a red color.
- Associated with production of strong acids.

Voges-Proskauer (VP)

- Glucose breakdown produces acetylmethyl carbinol; oxidation by air gives diacetyl, which reacts with alpha-naphthol under alkaline conditions (KOH) to produce red color.
- PPT lists 1 mL of 40% KOH and 3 mL alpha-naphthol.
- Associated with production of weak acids.
- PPT states organisms cannot produce strong and weak acid simultaneously; therefore MR+ / VP– and vice versa.

Citrate

- Use of citrate as sole carbon source allows ammonium ion accumulation and alkaline pH, producing blue color.
- Broth contains ammonium citrate and bromthymol blue.
- Koser's citrate agar corresponds to Simmons citrate in the PPT.

Urea / urease

- Some organisms secrete urease, which breaks down urea to ammonia and changes phenol red.
- PPT lists a 4-hour reading and Christensen's urea broth or agar slant.

Gelatin hydrolysis

- Detects ability to produce gelatinase.
- PPT uses it to distinguish gelatinase-positive S. aureus from negative S. epidermidis.
- Serratia and Proteus produce gelatinase.
- Organisms listed as positive: Bacillus anthracis, B. cereus, B. subtilis, Clostridium perfringens, and C. tetani.

Hydrogen sulfide (H2S)

- H2S reacts with ferric ammonium citrate to produce black color.
- Some bacteria degrade sulfur-containing amino acids such as cystine/cysteine, producing H2S that reacts with heavy metals such as lead or iron to form black precipitate.

Phenylalanine deaminase (PAD)

- Some organisms remove an amino group from phenylalanine, forming phenylpyruvic acid.
- The deaminase removes the amino group and releases ammonia.
- 10% ferric chloride is added.
- Green = positive; colorless/straw = negative.
- Phenylpyruvic acid reacts with ferric chloride to produce dark green.
- Differentiates Proteus, Morganella, and Providencia.

Lysine/ornithine decarboxylase

- Organisms can remove carboxyl groups from lysine, ornithine, or arginine.
- Decarboxylase broth contains nutrients, dextrose, pyridoxal, and pH indicators bromcresol purple and cresol red.
- Dextrose is the fermentable carbohydrate; pyridoxal is an enzyme cofactor.
- Bromcresol purple is purple at alkaline pH and yellow at acidic pH.
- Initial glucose fermentation turns the medium yellow.
- If the amino acid is decarboxylated, alkaline products raise pH and turn the broth purple.

- Arginine is hydrolyzed to ornithine and then decarboxylated to putrescine; lysine decarboxylation yields cadaverine.
- Yellow/no change = decarboxylase negative; purple = positive.
- Ornithine decarboxylase and arginine decarboxylase/dihydrolase are listed separately in the PPT.
- Decarboxylation/dihydrolation are anaerobic reactions, so tubes are overlaid with oil.
- Small amount of glucose initially turns broth yellow; later alkaline amino-acid products turn it purple.

ONPG

- Detects organisms producing beta-galactosidase.
- Beta-galactosidase breaks ONPG into nitrophenyl and galactose; nitrophenyl produces yellow color.
- A late lactose fermenter contains beta-galactosidase.
- Yellow = late lactose fermenter (LLF); no color = true NLF.

DNase

- Differential medium testing production of extracellular DNase.
- DNase agar contains nutrients, DNA, and methyl green.
- Methyl green binds negatively charged DNA.
- DNase-producing organisms hydrolyze DNA; fragments no longer bind methyl green, producing a clear halo around growth.

Motility

- Semi-solid medium demonstrates growth away from the stab line.

Carbohydrate fermentation

- Determines ability of microorganisms to ferment a specific carbohydrate.
- Detected by pH-indicator color change as acid products form.

O/F (oxidation/fermentation)

- Organisms requiring O2 as final hydrogen-ion acceptor are oxidative.
- Organisms unable to use glucose or other carbohydrates fermentatively without oxygen are nonfermenters.
- O/F medium has high carbohydrate concentration (1%) to enhance acid end-product production.
- Peptone is low (0.2%) so its utilization does not neutralize the acids.
- Acid in both open and closed tubes = organism is both oxidizer and fermenter.
- Acid only in closed tube = fermenter.
- Acid only in open tube = oxidizer.

24. HIGH-YIELD ORGANISM COMPARISON

Organism/group	Key PPT clues
E. coli	LF; pink MAC; motile; IMViC ++–+; urease –; glucose/mannitol/maltose with gas; major UTI and diarrheal pathogen
Shigella	NLF; nonmotile; glucose acid/no gas; dysentery; very low infective dose
Salmonella	Motile; NLF; H2S+; lactose/sucrose–; >2400 serologic types; typhoid/bacteremia/enterocolitis
Klebsiella	Nonmotile; capsule; mucoid; LF; urease+; citrate+; MR–/VP+; TSI A/A + gas
Enterobacter	Motile; colonies resemble Klebsiella; MR–/VP+
Serratia	Slow LF; pink pigment at room temperature; nosocomial infections; resistance
Proteus	Swarming; strong urease+; PAD+; burned-chocolate odor; lactose–
Morganella	M. morganii; UTI; urease+; PAD+
Providencia	Urinary/nosocomial; PAD+
Edwardsiella tarda	Motile; H2S+; glucose gas; lactose/sucrose–; indole/MR/Lys/Orn+; colistin intrinsic resistance
Yersinia	Safety-pin with special stains; nonmotile at 37°C; best growth 25–28°C; CIN target colonies for Y. enterocolitica

25. RAPID RECALL — TEST PRINCIPLES

- TSI → glucose first; lactose/sucrose at higher concentration; acid butt from glucose; unused slant peptones become alkaline.
- Nitrate reduction → red after reagents indicates nitrite; zinc red = true negative; zinc no-red = nitrate reduced beyond nitrite.
- Oxidase → cytochrome oxidase; indophenol = dark purple.
- Indole → tryptophanase; Kovac's reagent.
- MR → stable strong-acid end products; red.
- VP → acetoin/diacetyl pathway; alpha-naphthol + KOH; red.
- Citrate → citrate as sole carbon source; alkaline/blue.
- Urease → urea → ammonia; phenol red changes.
- Gelatinase → gelatin hydrolysis.
- H2S → black precipitate with iron/ferric compounds.
- PAD → phenylalanine → phenylpyruvic acid; ferric chloride → green.
- Decarboxylase → initial yellow from glucose, then purple if amino acid decarboxylated.
- ONPG → beta-galactosidase; yellow = late lactose fermenter.
- DNase → DNA hydrolysis; clear halo on methyl-green DNA agar.
- Motility → growth away from stab line.
- Carbohydrate fermentation → acid-induced indicator change.
- O/F → open only = oxidizer; closed only = fermenter; both = both.

26. EXAM-STYLE ASSOCIATIONS FROM THE PPT

- Pink MAC colony → lactose fermenter.
- Colorless MAC colony → non-lactose fermenter.
- Mucoid colony + nonmotile + capsule → think Klebsiella.
- Swarming + strong urease + PAD → think Proteus.
- Pink pigment at room temperature → think Serratia marcescens.

- Burned-chocolate odor → Proteus.
- K1 capsule → neonatal E. coli meningitis/septicemia.
- LT → Gs → adenylate cyclase → cAMP.
- ST → cGMP.
- O157:H7 → EHEC/STEC and HUS association in the PPT.
- Stacked-brick HEp-2 adherence → EAEC.
- Shigella → nonmotile, NLF, dysentery, low infectious dose.
- S. typhi → typhoid fever; human-only reservoir; rose spots; blood culture early, stool culture later; gallbladder carrier state.
- S. typhi → negative citrate and ornithine decarboxylase according to the PPT.
- Edwardsiella tarda → H2S+, indole+, colistin intrinsic resistance.
- Yersinia → safety-pin appearance with Wayson/Wright/Giemsa; best growth 25–28°C.
- Y. enterocolitica → CIN target colony; cold enrichment; motile at 25°C but not 37°C.
- Phenylalanine deaminase positive → Proteus/Morganella/Providencia.
- ONPG yellow → late lactose fermenter.
- Zinc + red after nitrate test → true negative.
- Zinc + no red → positive nitrate reduction beyond nitrite to nitrogen gas.

27. FINAL CRAM SHEET

- ENTEROBACTERIACEAE: GNB, facultative anaerobes, non-sporing, usually oxidase–, glucose fermenters, nitrate reducers; normal GI flora and major nosocomial pathogens.
- MAC: LF = pink; NLF = colorless.
- E. COLI: pink MAC, motile, IMViC ++–+, urease–, UTI/neonatal meningitis/diarrhea.
- EPEC: infantile diarrhea; brush-border disruption; HEp-2 adherence.
- ETEC: LT/ST; traveler's diarrhea; watery diarrhea.
- EIEC: invasive; Shigella-like; dysentery.
- EHEC: Shiga-like toxin; hemorrhagic colitis/HUS; O157:H7.
- EAEC: stacked-brick adherence; persistent diarrhea.
- DAEC: pediatric diarrhea and UTI associations.
- KLEBSIELLA: capsule, mucoid, nonmotile, LF, urease+, citrate+, MR–/VP+.
- ENTEROBACTER: motile, MR–/VP+.
- SERRATIA: slow LF, pink pigment.
- PROTEUS: swarming, urease+, PAD+, lactose–.
- SALMONELLA: motile, NLF, H2S+, typhoid/bacteremia/enterocolitis.
- SHIGELLA: nonmotile, NLF, acid/no gas, dysentery, very low infective dose.
- EDWARDSIELLA TARDA: H2S+, indole+, glucose gas, Salmonella-like, colistin intrinsic resistance.
- YERSINIA: safety pin, special-stain bipolarity, slow growth, 25–28°C; CIN for Y. enterocolitica.
- TSI: know slant/butt logic.
- NITRATE: zinc red = negative; zinc no-red = positive beyond nitrite.
- MR = strong acids; VP = weak-acid/acetoin pathway.
- PAD = green with ferric chloride.

• ONPG yellow = late lactose fermenter.

• DNase = clear halo.

• O/F: open+closed = both; closed only = fermenter; open only = oxidizer.

END OF COMPLETE PPT-BASED REVIEWER

Source basis: uploaded Enterobacteriaceae.pptx. No outside web material was used to fill the reviewer.