THE ENTEROBACTERIACEAE

• **Reading Assignment:** Murray, et. al., *Medical Microbiology*, 8th ed., 2016, Chapter 25.

The Enterobacteriaceae

**OBJECTIVES**

1. Describe diseases associated with Shigella, Salmonella, Citrobacter, Klebsiella, Enterobacter, Serratia, Proteus and Yersinia
2. Compare the transmission and clinical manifestations of E. coli, Shigella, Salmonella and Y. enterocolitica
3. Describe the pathogenesis of Shigella, Salmonella, and Yersinia
4. List clinical manifestations of Salmonella infection
5. Describe tools used in the laboratory to aid in isolation and identification of Enterobacteriaceae
Shigella

- Important species
  - *Shigella dysenteriae* – serogroup A
  - *Shigella flexneri* – serogroup B
  - *Shigella boydii* – serogroup C
  - *Shigella sonnei* – serogroup D

ABCD=Dirty Fingers Bring Shigella

- *Shigella sonnei* accounts for 70% of U.S. isolates

Shigella

Key Characteristics

- Biochemically, antigenically, and genetically to indistinguishable from *E. coli*
- Unlike *E. coli*, *Shigella* are
  - Non-lactose fermenters
  - Non-gas producers
  - Non-motile
Shigella
Reservoir and Transmission
• Person to person via fecal oral route
• Reservoir: Intestines of humans?
• 500,000 cases reported annually in USA
• Contaminated water, food, flies, fingers, fomites, feces (5 F’s)
• Highest risk
  – Young children in day care center, nurseries, custodial institutions
  – Siblings and parents of these children
  – MSM

Shigella
Clinical Syndromes
• Bacillary Dysentery
  – Abdominal cramps, tenesmus, pus and blood in stool
  – Tissue invasion limited to epithelial cells and submucosa
  – Fecal leukocytes present
  – Incubation 1-3 days lasting 48 hrs
  – Most communicable of bacterial diarrheas
  – 10-100 viable organisms can produce disease—different from Salmonella
  – Concentration in stools is $10^3 - 10^4$ CFU/gram

Shigella
Pathogenesis
• Virulent strains carry plasmid for attachment and entry
• Enters cells by phagocytic vacuole
• Organism escapes in cytoplasm
• Intracellular replication
• Actin “tail” drives organism in cytoplasm
• Organisms enter adjacent cells
**Shiga toxin**

- *S. dysenteriae* strains produce exotoxin
- Compare to toxin produced by *E. coli* (STEC—shiga toxin producing *E. coli*)
  - Stx1 is identical
  - Stx2 60% homology
- Disrupts protein synthesis in the cell and leads to epithelial cell damage

**Salmonella**

**Nomenclature**

Important species:
- *Salmonella enterica*
  - all serotypes considered single species except *S. bongori*
- Certain serotypes listed as species
  - *S. typhi/paratyphi*
  - *S. typhimurium*
  - *S. enteritidis*

**Salmonella**

**Key Characteristics**

- Non-lactose fermenter
- Produces hydrogen sulfide gas
Salmonella
Reservoir and Transmission

- Lower animals (poultry, cows, pigs, reptiles, birds) for non-typhoid strains.
- Humans for *S. typhi* (establish chronic carriage in gall bladder)
- Transmission, ingestion via improper food handling
  - About half of Salmonella epidemics are result of contaminated poultry and poultry products
  - Contaminated cutting board used in preparation
- Secondary transmission person-to-person
- Requires high inoculum for disease, except for *S. typhi*

Salmonella
Incidence

- Estimated 1.4 million cases of non-typhoidal salmonellosis occur in U.S. annually; 16,000 hospitalizations and 600 deaths.
- Outbreaks often associated with chickens or reptiles
- ~500 cases of *S. typhi* reported in US each year—associated with travel
  - 27 million cases and 200,000 deaths worldwide

2017 CDC Notes Backyard Flocks Blamed in Salmonella Outbreaks

- 961 people
  - 30% kids under age 5
  - 10 separate outbreaks of salmonella
- Recover within a week without treatment
- More likely to be severe among young children, the elderly, and people with weakened immune systems
- CDC’s recommendations to stay safe:
  - Wash your hands after handling live poultry
  - Don’t let the animals into your house
  - Don’t let kids under age 5 touch live poultry or eggs without supervision
Salmonella
Clinical Syndromes

• Asymptomatic (carrier) state —
  – Relatively limited with non-S. typhi occasionally prolonged over a year.
  – Chronic or decades long with S. typhi

• Febrile Gastroenteritis - most common presentation.
  – Found in 2/3 of patients with culture confirmed Salmonella infections. Incubation 12-48 h.
  – Symptoms include malaise, nausea, sometimes with vomiting, followed by abdominal pain and diarrhea. Self-limited. Duration 3-5 days.

Salmonella
Clinical Syndromes

• Septicemia without major gastrointestinal involvement —
  – Characteristically found in patients with underlying conditions, leukemia, lymphoma, AIDS, SLE, sickle cell crisis, and alcoholic hepatitis.
  – Increased risk in pediatric, geriatric and AIDS patients
  – Clinically similar to other Gram negative bacteremia
  – 10% localized suppurative infections
    • Osteomyelitis, endocarditis, arthritis
  – Diagnosed by positive blood cultures

Salmonella
Clinical Syndromes

• Enteric Fever —
  – Typhoid Fever is best known example
  – Milder form known as Paratyphoid fever
  – Caused by S. typhi and S. paratyphi A and B, rare with other serotypes.
  – Travel history can aid in diagnosis
  – Incubation 1-2 weeks. Length of illness is 4 weeks characterized by increasing fever for 2 weeks (bacteremic stage) followed by gastrointestinal symptoms for 1-2 weeks.
  – Presents with fever, headache, rose spots, constipation
  – Rose spots; pink macules or pruritic lesions
**Pathogenesis of Gastroenteritis**

- Salmonella adhere and stimulate rearrangement of plasma cell membrane to form ruffles
- Bacteria are engulfed and enter cell in vesicles
- Organisms are translocated across epithelial cells of large and small bowel and are presented to macrophages along lamina propria
- Large inflammatory response induced

**Pathogenesis of Enteric Fever**

- Bacteria bind to M cells
- Bacteria go to Peyer’s patches, primary area for gut to present antigens to immune system
- Organisms invade macrophages and multiply
- Inhibition of oxidative metabolic burst allows organisms to survive intracellularly
- Capsular polysaccharide allows for evasion of immune system
- Bacteria spread via macrophage to spleen, lymph nodes, bloodstream
- Colonization of the gallbladder
- Transmitted via stool

**Citrobacter**

**Overview**

- Important species:
  - C. freundii
  - C. koseri
- Habitat: Intestinal tract humans/animals
- Clinical Syndromes:
  - Nosocomial infections of urinary and respiratory tracts of debilitated, hospitalized patients, endocarditis and hospital acquired bacteremia
  - C. freundii - rare cause of diarrhea
  - C. koseri - rare cause of meningitis and brain abscess in neonates
- Different antimicrobial susceptibility profiles
Klebsiella
Overview

- Important species:
  - K. pneumoniae
  - K. oxytoca
- Habitat: Intestines and upper respiratory tract of humans and animals
- Clinical Syndromes:
  - Primary lobar pneumonia characterized by destructive changes, necrosis, and hemorrhage ("currant jelly" sputum), bronchopneumonia, bronchitis,
  - Accounts for 9% of UTI's and 14% of bacteremia in hospitalized patients
- Capsule is a virulence factor

Enterobacter
Overview

- Important species:
  - E. aerogenes
  - E. cloacae
- Habitat: Widely distributed in the environment and GI tract of humans
- Clinical Syndromes:
  - Frequent colonizers of hospital patients, cause opportunistic infections involving urinary tract, respiratory tract, cutaneous wounds, occasional cause of septicemia and meningitis
- Antibiotic therapy can be ineffective, because the organisms are frequently resistant to multiple antibiotics

Serratia
Overview

- Important species:
  - S. marcescens
- Habitat: Widely distributed in the environment
- Clinical Syndromes:
  - Nosocomial infections, pneumoniae, septicemia, UTI, surgical wound and cutaneous infections. Also reported as a cause of endocarditis and osteomyelitis in IV drug addicts
- Key Features
- Production of deep red pigment called prodigiosin
- Very interesting history

Serratia Infections: from Military Experiments to Current Practice.
S. Mahlen, CMR, 2011
Overview

• Important species:
  – *P. mirabilis*
  – *P. vulgaris*
• Habitat: Soil, water and intestinal tract of humans and animals.
• Clinical Syndromes:
  – UTI and wound infections
• Key features:
  – Strongly urease positive produces highly alkaline urea which can lead to renal calculi composed of struvite (magnesium ammonium phosphate)
  – Swarming observed on blood agar

Yersinia

Overview

• Important species:
  – *Yersinia pestis* (discussed with zoonotic infections)
  – *Yersinia enterocolitica*
• Habitat: Widely distributed in lakes and reservoirs; Pigs are a major reservoir for infection in humans; milk and water are also sources
• Clinical syndromes:
  – Diarrheal illness associated with household preparation of chitterlings
• Portal of entry is oral digestive route
• Common in cold months
Chitterlings

- Chitterlings (sometimes spelled chitlins or chittlins in vernacular) are the viscera intestines of a pig that have been prepared as food.
- In various countries such food is prepared and eaten either as part of a daily diet, or at special events, holidays or religious festivities.

Yersinia enterocolitica

Clinical Syndromes

- Enterocolitis accounts for 2/3 of infection.
  - Average 4-6 days of incubation
  - Diarrhea, fever, abdominal pain lasting as long a 1-2 weeks.
- Chronic form can persist months to > year
  - Organism adheres to and penetrates the ileum, causing terminal ileitis, lymphadenitis, and acute enterocolitis.
  - Mimics appendicitis. Most common in children =pseudoappendicitis

Yersinia enterocolitica

Clinical Syndromes

- Associated w/transfusion related sepsis, arthritis, intraabdominal abscess, hepatitis, osteomyelitis
- Blood contamination occurs do to asymptomatic Y. enterocolitica bacteremia at time of blood donation
  - Can proliferate in blood stored at 4°C after 2-3 weeks
Lab Diagnosis

- Biochemicals
- Structural Properties (surface antigens)

Hektoen Agar

- Selective and differential
- Isolation and differentiation of enteric pathogens from clinical specimens
- Bile salts and dyes inhibit most Gram-positive organisms and most of the nonpathogenic coliform flora of the intestinal tract
- Production of H2S can be detected due to the addition of thiosulfate and ferric ammonium citrate
- Lactose, sucrose, salicin fermentation

Stool culture on Hektoen enteric agar: mixed flora including Escherichia coli (red arrow), Salmonella (blue arrow), and Proteus vulgaris (yellow arrow).

https://microbeonline.com/hektoen-enteric-agar-composition-principle-uses/
Hektoen Agar

H2S Producers: Salmonella, Citrobacter, Proteus
Non-Lactose Fermenters: Salmonella, Shigella, Proteus, Citrobacter

Klebsiella capsule

Urease
- Many organisms, especially those that infect the urinary tract
- Split urea in the presence of water to release ammonia and carbon dioxide.
- Mnemonics to remember urease positive organisms: **PUNCH**
  - P: Proteus
  - U: Ureaplasma
  - N: Nocardia
  - C: Cryptococcus neoformans
  - H: Helicobacter pylori

Serotyping

- Latex agglutination
- Identify presence of different capsule, cell, and flagellar antigens to “identify” the serotype of the organism
- Ab-Ag interaction
  - O antigen
  - H antigen

BioFire FilmArray – Multiplex PCR

- Integrated Sample Preparation, Amplification, and Detection for 22 Enteric Pathogens including:

FilmArray™ Gastrointestinal Panel – Multiplex PCR – One Test: 22 Results

- Campylobacter (jejuni, coli and upsaliensis)
- Clostridium difficile
- Salmonella
- Yersinia enterocolitica
- Vibrio (parahaemolyticus, vulnificus and cholerae)
- Shiga-like toxin-producing E. coli (ETEC)
- E. coli O157
- Shigella/Enteroinvasive E. coli (EIEC)
- Enterotoxigenic E. coli (ETEC)
- Enteropathogenic E. coli (EPEC)
- Diarrheogenic E. coli (EaggEC)
- Shiga-toxin-producing Enterohemorrhagic E. coli (STEC)
- Diarrheagenic E. coli/Shigella
- Enteropathogenic E. coli (EPEC)
- Enterotoxigenic E. coli (ETEC)
- Parasites
  - Cryptosporidium
  - Cyclospora cayetanensis
  - Entamoeba histolytica
  - Giardia lamblia
- Viruses
  - Adenovirus
  - Astrovirus
  - Norovirus/GI/GII
  - Rotavirus A
  - Sapovirus (I, II, IV and V)
A 50 year old female returned to Chicago from a 3 week trip to Jakarta (Indonesia) and about 5 days later started having flu-like symptoms and a low-grade fever. On the day prior to admission she had spiking fevers followed by profuse sweating. Stool cultures, ova and parasite exams and urine cultures were all negative. The blood culture grew a glucose-fermenting, gram-negative rod that was oxidase negative. Additional testing showed the organism was lactose-negative and H₂S positive. What is the likely etiologic agent of this patient’s symptoms?

A. Shigella  
B. E. coli O157  
C. Malaria (Plasmodium falciparum)  
D. Salmonella typhi  
E. Vibrio cholera

A 1-year-old patient was brought into the emergency room of an intercity hospital suffering from diarrhea and subsequent dehydration. The family reports that the illness began a couple of days after Christmas. In questioning the parents about food-born source of the infection the parents reported serving chitterlings to the adults but not the children. A stool specimen sent to the laboratory grew a lactose-negative gram-negative bacillus that gave biochemical reactions typical of E. coli except that the organism was urease positive. What is the most likely identity of the bacterium?

A. E. coli 0157:H7  
B. Salmonella serotype arizonae  
C. Yersinia enterocolitica  
D. Vibrio cholerae  
E. Edwardsiella tarda