Introduction to Microbiology

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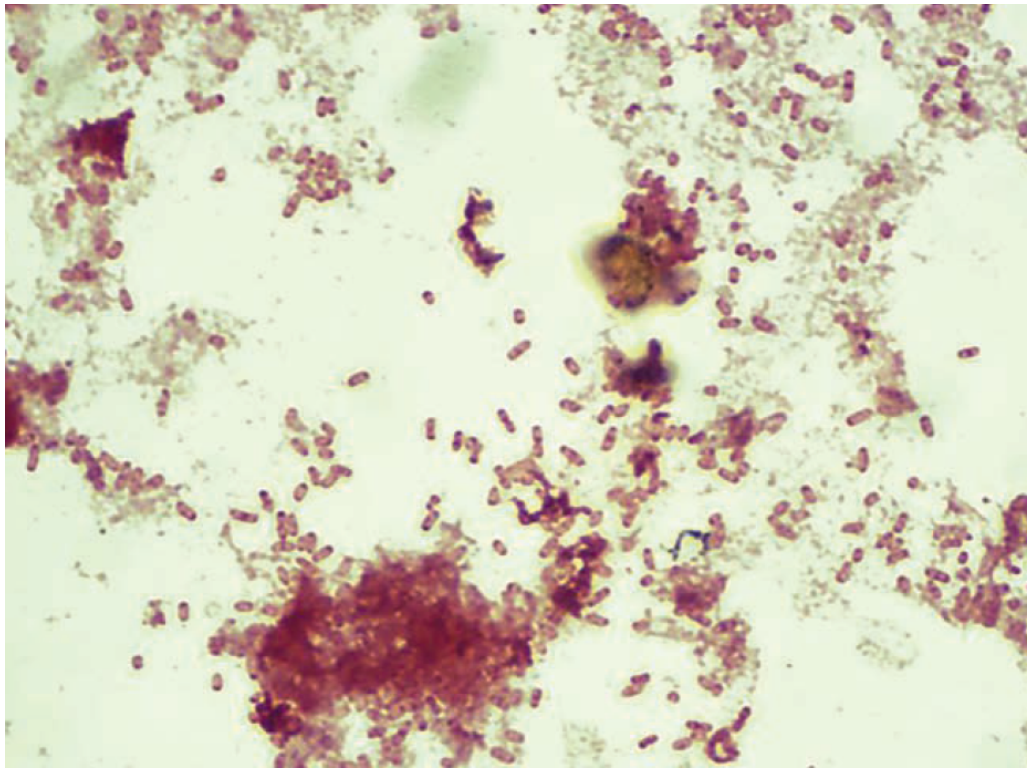
ENTEROBACTERIACEAE / Classification

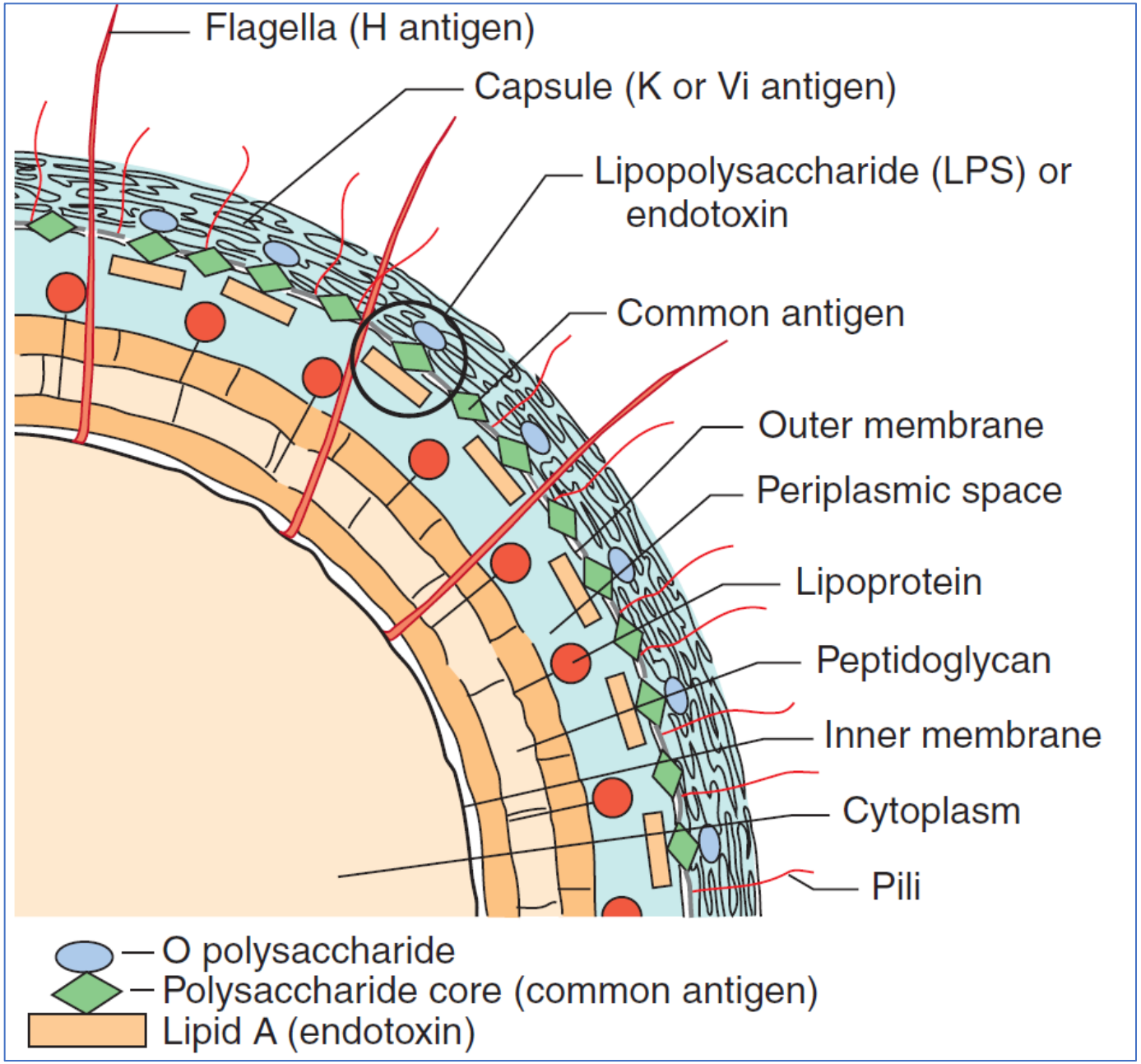
- More than 50 genera and hundreds of species and subspecies, Enterobacteriaceae are **ubiquitous (**organisms found worldwide in soil, water, and vegetation and are part of the normal intestinal flora of most animals, including humans)**.**

- In humans it can be part of the **normal intestinal flora**, or **always associated with human disease, or opportunistic infections, or** normally commensal organisms that become pathogenic when they acquire virulence genes.

- Enterobacteriaceae are moderate-sized (0.3 to 1.0 × 1.0 to 6.0 µm), **non–spore-forming, gram-negative rods** , **facultative anaerobes** that share a common antigen **enterobacterial common antigen**

Scientific classification	
Domain:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacterales
Family:	**Enterobacteriaceae** Rahn, 1937




Flagella (H antigen)
Capsule (K or Vi antigen)
Lipopolysaccharide (LPS) or endotoxin
Common antigen
Outer membrane
Periplasmic space
Lipoprotein
Peptidoglycan
Inner membrane
Cytoplasm
Pili
O polysaccharide
Polysaccharide core (common antigen)
Lipid A (endotoxin)

ENTEROBACTERIACEAE / Pathogenesis and Immunity

- **Endotoxin** toxin activity depends on the **lipid A** component of LPS, released at cell lysis.
- **Capsule,** hydrophilic capsular antigens repel the hydrophobic phagocytic cell surface, but anticapsular antibodies diminish the capsule role.
- **Antigenic Phase Variation,** somatic O antigens, capsular K antigens, and flagellar H antigens alternately expressed or not expressed (phase variation).
- **Type III Secretion Systems.**
- The bacteria counteract **iron sequestration** by producing their own competitive **siderophores** or iron-chelating compounds (e.g., **enterobactin, aerobactin**). Or from Iron released from lysed cells.
- Resistance to Serum Killing and Antimicrobial Resistance.

ENTEROBACTERIACEAE/MacConkey's agar

- It contains **bile salts** (to inhibit most Gram-positive bacteria), **crystal violet dye** (which also inhibits certain Gram-positive bacteria), **neutral red dye** (which turns pink if the microbes are fermenting lactose).

- Enterobacteriaceae are classified based on biochemical properties, antigenic structure, and molecular analysis of their genomes, protein composition by mass spectrometry.

MacConkey's agar showing both lactose and non-lactose fermenting colonies. Lactose fermenting colonies are pink whereas non-lactose fermenting ones are colourless or appear same as the medium.

ENTEROBACTERIACEAE/ Antibiotic resistance

- Resistance of the Enterobacteriaceae to antibiotics, especially of the β lactam type, is increasingly dominated by the mobilization of continuously expressed single genes that encode efficient drug modifying enzymes.

- **Multi drug resistant** (MDR) Enterobacteriaceae has been frequently reported from different parts of the world as an emergence of treatment problem. Antibiotics given empirically without proper antibiotic susceptibility testing are one of the major causes for the development of MDR.

- There is a shift of the "natural" resistance, such as **membrane impermeability** and **drug efflux**, to the modern paradigm of **mobile gene pools** that transmit resistance between bacteria.

- https://www.bmj.com/content/352/bmj.h6420

ENTEROBACTERIACEAE / overview

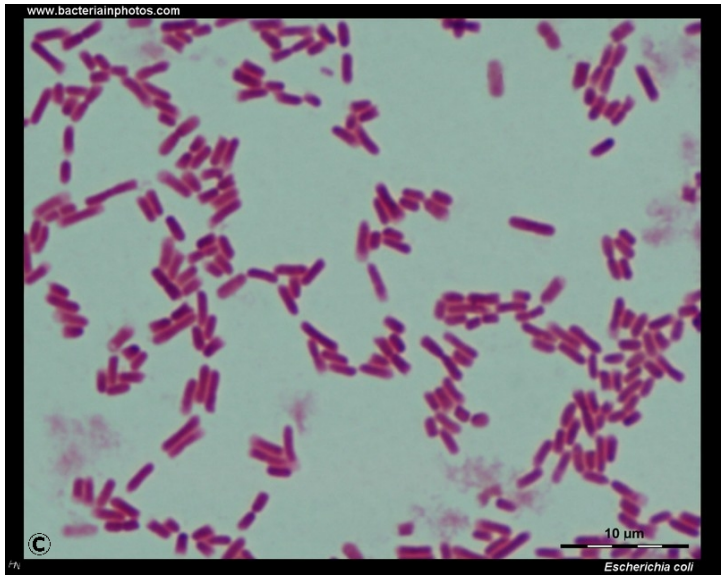
The following pathogens are discussed

- ***Escherichia coli***
- **Salmonella**
- **Shigella**
- **Yersinia**
- **Klebsiella**
- **Proteus**

Escherichia coli

- *E. Coli* is the model organism of *Enterobacteriaceae* since it is the most extensively studied.

- *E. coli* is the most common and important member of the genus *Escherichia*.



- It is both a common **commensal inhabitant of the gastrointestinal tract** and one of the **most important pathogens** in humans.

- It is a frequent cause of diahrrheal disease.

- It is the most frequent cause of **bloodstream infection** and **urinary tract infections** (UTIs) among **Gram-negative bacteria.**

Escherichia coli strains

- **Commensal strains innocuously colonize the colon of healthy hosts**, causing extraintestinal disease only in the presence of a large inoculum (e.g., with penetrating abdominal trauma) and/or significant host compromise.

- **Diarrhoeagenic strains cause diarrhoea syndromes** that vary in clinical presentation and pathogenesis according to the strain's distinctive virulence traits

- **Extraintestinal pathogenic *E. coli*** (ExPEC) often innocuously colonize the human gut. However, they have a unique ability to enter and **survive within normally sterile extraintestinal body** sites, and to **cause disease** when they do so.

Organism	Site of Action	Disease	Pathogenesis
Enterotoxigenic *E. coli* (ETEC)	Small intestine	Traveler's diarrhea; infant diarrhea in developing countries; watery diarrhea, vomiting, cramps, nausea, low-grade fever	Plasmid-mediated, heat-stable (ST) and heat-labile (LT) enterotoxins that stimulate hypersecretion of fluids and electrolytes
Enteropathogenic *E. coli* (EPEC)	Small intestine	Infant diarrhea in developing countries; watery diarrhea and vomiting, nonbloody stools; believed to be rare in United States	Plasmid-mediated A/E histopathology, with disruption of normal microvillus structure resulting in malabsorption and diarrhea
Enteroaggregative *E. coli* (EAEC)	Small intestine	Infant diarrhea in developing and probably developed countries; traveler's diarrhea; persistent watery diarrhea with vomiting, dehydration, and low-grade fever	Plasmid-mediated aggregative adherence of rods ("stacked bricks") with shortening of microvilli, mononuclear infiltration, and hemorrhage; decreased fluid absorption
Shiga toxin–producing *E. coli* (STEC)	Large intestine	Initial watery diarrhea followed by grossly bloody diarrhea (hemorrhagic colitis) with abdominal cramps; little or no fever; may progress to hemolytic uremic syndrome	STEC evolved from EPEC; A/E lesions with destruction of intestinal microvilli, resulting in decreased absorption; pathology mediated by cytotoxic Shiga toxins (Stx1, Stx2), which disrupt protein synthesis
Enteroinvasive *E. coli* (EIEC)	Large intestine	Rare in developing and developed countries; fever, cramping, watery diarrhea; may progress to dysentery with scant bloody stools	Plasmid-mediated invasion and destruction of epithelial cells lining colon

Enterotoxigenic E. coli (ETEC)

- one of the most common causes of bacterial diarrheal disease in developing countries, and 30% of **traveler's diarrhea*.Acquired through consumption of fecally contaminated food or water.** Person-to-person spread does not occur.

- 1- to 2-day incubation period and persists for an average of 3 to 5 days.

- The symptoms: **Secretory diarrhea** (watery, non-bloody diarrhea) and **abdominal cramps**; less commonly nausea and vomiting. Can be fatal in undernourished individuals.

- Produces 2 classes of toxins:

Heat stable toxin leads to **increase in cyclic guanosine monophosphate (cGMP)** and subsequent hypersecretion of fluids well as inhibition of fluid absorption
heat labile toxins leads to **increase in cyclic adenosine monophosphate (cAMP)** levels, resulting in enhanced secretion of chloride and decreased absorption of sodium and chloride

***traveler's diarrhea :** When you visit a place where the climate or sanitary practices are different from yours at home, you have an increased risk of developing traveler's diarrhea. Usually recovery happens within days with no need for treatment.

Enterotoxigenic E. coli (ETEC)

A second-year medical student experiences **watery diarrhea** and mild **abdominal cramps** during his 2-week travel to Egypt. With his little medical knowledge, he makes several assumptions, which of those assumption is **false**?

a) This is probably a case of traveler's diarrhea that should resolve within a few days.
b) Enterotoxigenic *E. coli* (ETEC) is a probable causative agent.
c) He would not have become sick if he washed his hands properly.
d) Liquids are important to prevent dehydration and loss of electrolytes.
e) If it is traveler's diarrhea, he probably contracted the pathogen in a meal he ate 2 days ago.

Shiga toxin–producing E. coli (STEC)

- Most infections are attributed to the consumption of undercooked meat products, water, unpasteurized milk or fruit juices uncooked vegetables, and fruits. **Ingestion of fewer than 100 bacteria can produce disease,** and person-to-person spread occurs.

- Disease caused by STEC ranges from mild uncomplicated diarrhea to **hemorrhagic colitis** with severe abdominal pain and bloody diarrhea. Severe disease is more commonly associated with **STEC O157:H7**.

- 3 to 4 days of incubation, Within 2 days of onset, disease in 30% to 65% of patients progresses to a bloody diarrhea with severe abdominal pain, Complete resolution of symptoms typically occurs after 4 to 10 days in most untreated patients.

- **Hemolytic uremic syndrome (HUS),** a disorder characterized by acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia, is a **complication** in 5% to 10% of infected children younger than 10 years.

Clinical Case 25-1 Multistate Outbreak of Shiga Toxin–Producing *Escherichia coli* (STEC) Infections

In 2006, *E. coli* 0157 was responsible for a large multistate outbreak of gastroenteritis. The outbreak was linked to contamination of spinach, with a total of 173 cases reported in 25 states, primarily over an 18-day period. The outbreak resulted in hospitalization of more than 50% of the patients with documented disease, a 16% rate of hemolytic uremic syndrome, and one death. Despite the wide distribution of the contaminated spinach, publication of the outbreak and the rapid determination that spinach was responsible resulted in prompt removal of spinach from grocery stores and termination of the outbreak. This outbreak illustrates how contamination of a food product, even with small numbers of organisms, can lead to a widespread outbreak with a particularly virulent organism, such as strains of STEC.

Shiga-Toxin *E. coli* Hemolytic Uremic Syndrome: Review of Management and Long-term Outcome

https://link.springer.com/article/10.1007/s40124-020-00208-7

Escherichia coli/ Extraintestinal Infections

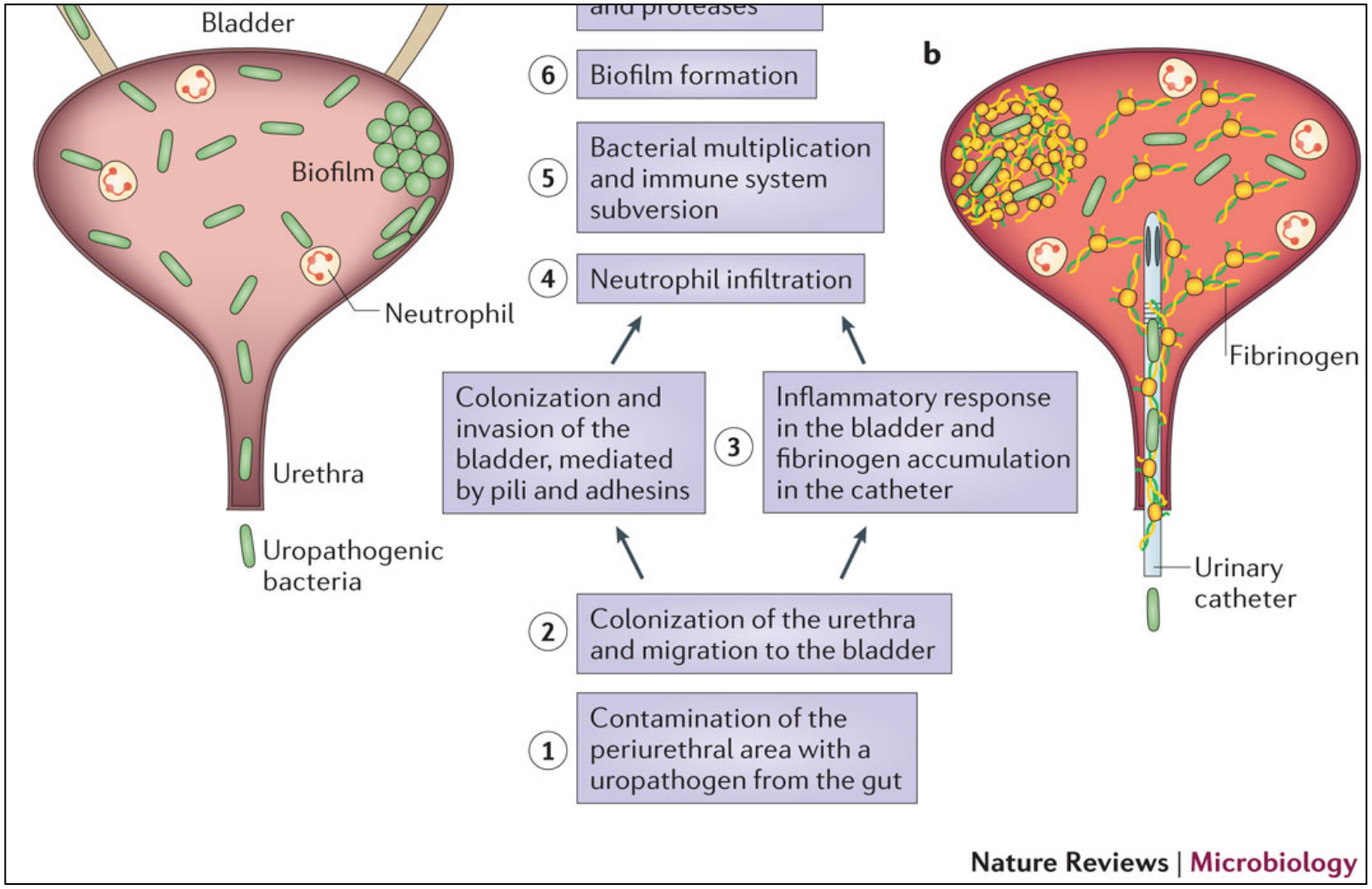
- **Urinary Tract Infection:** Most gram-negative rods that produce UTIs originate in the colon, contaminate the urethra, ascend into the bladder, and may migrate to the kidney or prostate.

- Almost every second woman suffers from a bladder infection at some point in her life (**E. coli in 80% of UTI cases**). Also men are affected by cystitis, though less frequently, probably due to anatomical differences (e.g. shorter urethra in women makes it easier for bacteria to reach the bladder)**.**

- **Neonatal Meningitis** : *E. coli* and group B streptococci cause the majority of CNS infections in infants younger than 1 month.

- **Septicemia** *:* Typically, septicemia caused by gram-negative rods, such as *E. coli,* most commonly originates from infections in the urinary or GI tract, with high mortality in immunocompromised patients.

Escherichia coli/ Extraintestinal Infections



Salmonella

- *Salmonella* can colonize virtually all animals (especially poultry)**.** Serotypes such as *Salmonella* Typhi and *Salmonella* Paratyphi are highly **adapted to humans** and do not cause disease in nonhuman hosts.

- After ingestion and passage through the stomach, salmonellae attach to the mucosa of the **small intestine** and invade into the **M (microfold) cells** located in Peyer patches, as well as into enterocytes. **The bacteria remain in endocytic vacuoles, where they replicate**. The bacteria can also be transported across the cytoplasm and released into the blood or lymphatic circulation. The inflammatory response confines the infection to the GI tract, mediates the release of prostaglandins, **and stimulates cAMP and active fluid secretion**.

- Virulence dependent on **pathogenicity island** on the bacterial chromosome. Encoding for toxins, attachment proteins and immune evasion mechanisms.

Salmonella

- **Asymptomatic Colonization :**

The strains of *Salmonella* responsible for causing typhoid and paratyphoid fevers are maintained by human colonization.

Mary Mallon

Cook

Mary Mallon, also known as Typhoid Mary, was an Irish cook. She was the first person in the United States identified as an asymptomatic carrier of the pathogen associated with typhoid fever. She was presumed to have infected 51 people, three of whom died, over the course of her career as a cook. Wikipedia

Born: September 23, 1869, Cookstown, United Kingdom

Died: November 11, 1938, Riverside Hospital

Salmonella / Epidemiology and diseases

- The most common sources of human infections are **poultry, eggs, dairy products,** and foods prepared on contaminated work surfaces, **large inoculum** (e.g., 10^6 to 10^8 bacteria) is required for symptomatic disease.

- The infectious dose for *Salmonella* Typhi infections **is low**, so **person-to-person spread is common**, occur when food or water contaminated by infected food handlers is ingested.

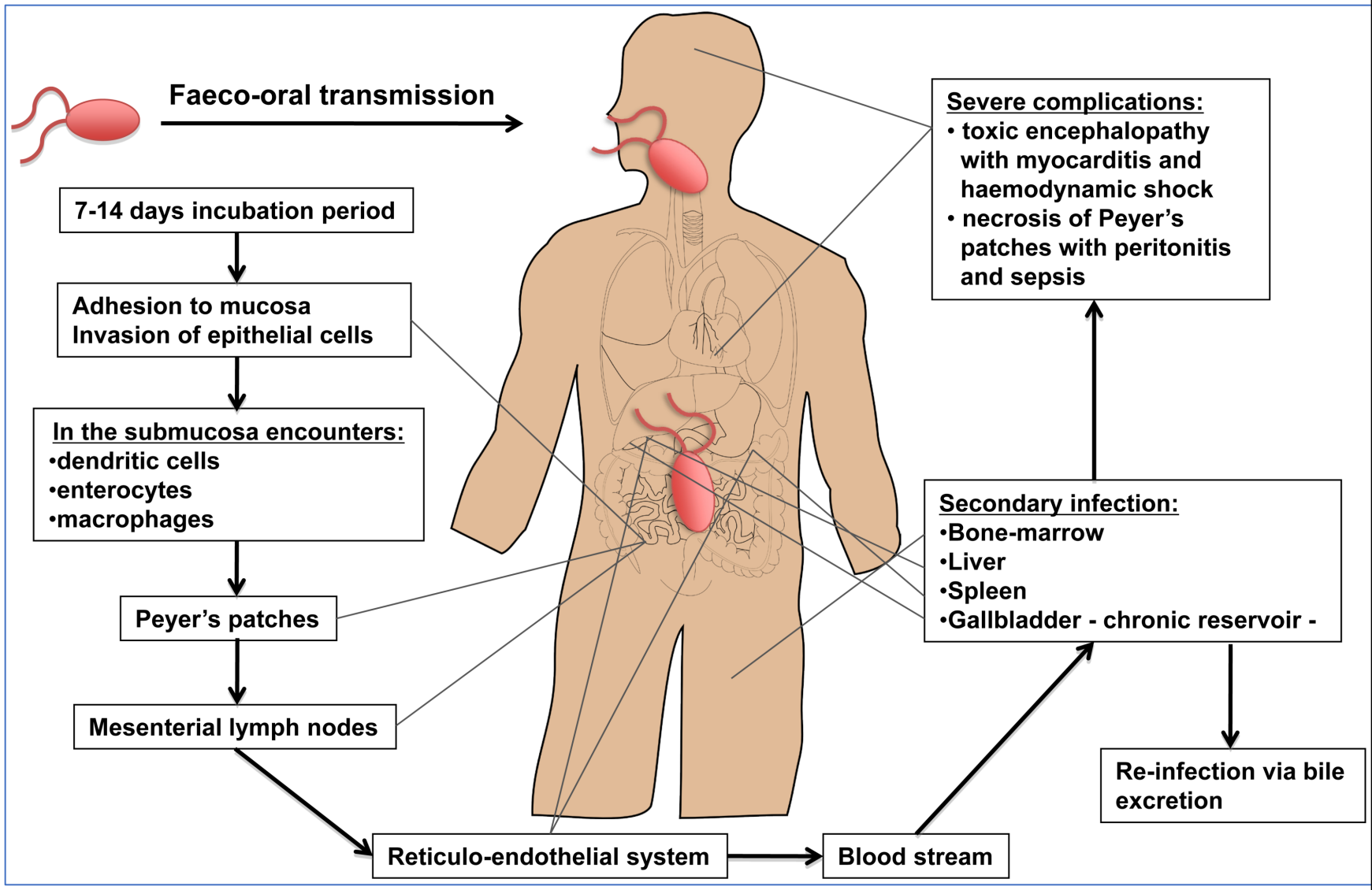
- **Gastroenteritis** is a **common form of salmonellosis, nausea, vomiting, and nonbloody diarrhea.** can persist for 2 to 7 days before spontaneous resolution.

- **Septicemia** All *Salmonella* species can cause bacteremia, although infections with *Salmonella* Typhi*, Salmonella* Paratyphi more commonly lead to a bacteremic phase.

Salmonella / diseases

- *Salmonella* Typhi produces a febrile illness called **typhoid fever.** A milder form of this disease, referred to as **paratyphoid fever,** is produced by other *Salmonella (e.g paratyphi).*

- The bacteria responsible for enteric fever pass through the cells lining the intestines and are engulfed by macrophages. They replicate after being transported to the liver, spleen, and bone marrow. Ten to 14 days after ingestion of the bacteria, patients experience gradually increasing fever, with nonspecific complaints of headache, myalgias, malaise, and anorexia).



Salmonella / diseases

Clinical Case 25-2 *Salmonella* Typhi Infection

Scully and associates (*N Engl J Med* 345:201–205, 2007) described a 25-year-old woman who was admitted to a Boston hospital with a history of persistent fever that did not respond to amoxicillin or acetaminophen or ibuprofen. She was a resident of the Philippines who had been traveling in the United States for the previous 11 days. On physical examination, she was febrile and had an enlarged liver, abdominal pain, and an abnormal urinalysis. Blood cultures were collected upon admission to the hospital and were positive the next day with *Salmonella* Typhi. Because the organism was susceptible to fluoroquinolones, this therapy was selected. Within 4 days, she had defervesced and was discharged to return home to the Philippines. Although typhoid fever can be a very serious life-threatening illness, it can initially present with nonspecific symptoms, as was seen in this woman.

Typhoid Fever

https://www.nejm.org/doi/full/10.1056/nejmra020201

Shigella

- *S. dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei.* However, analysis of DNA has determined that these four species are actually biogroups within the species ***E. coli.***

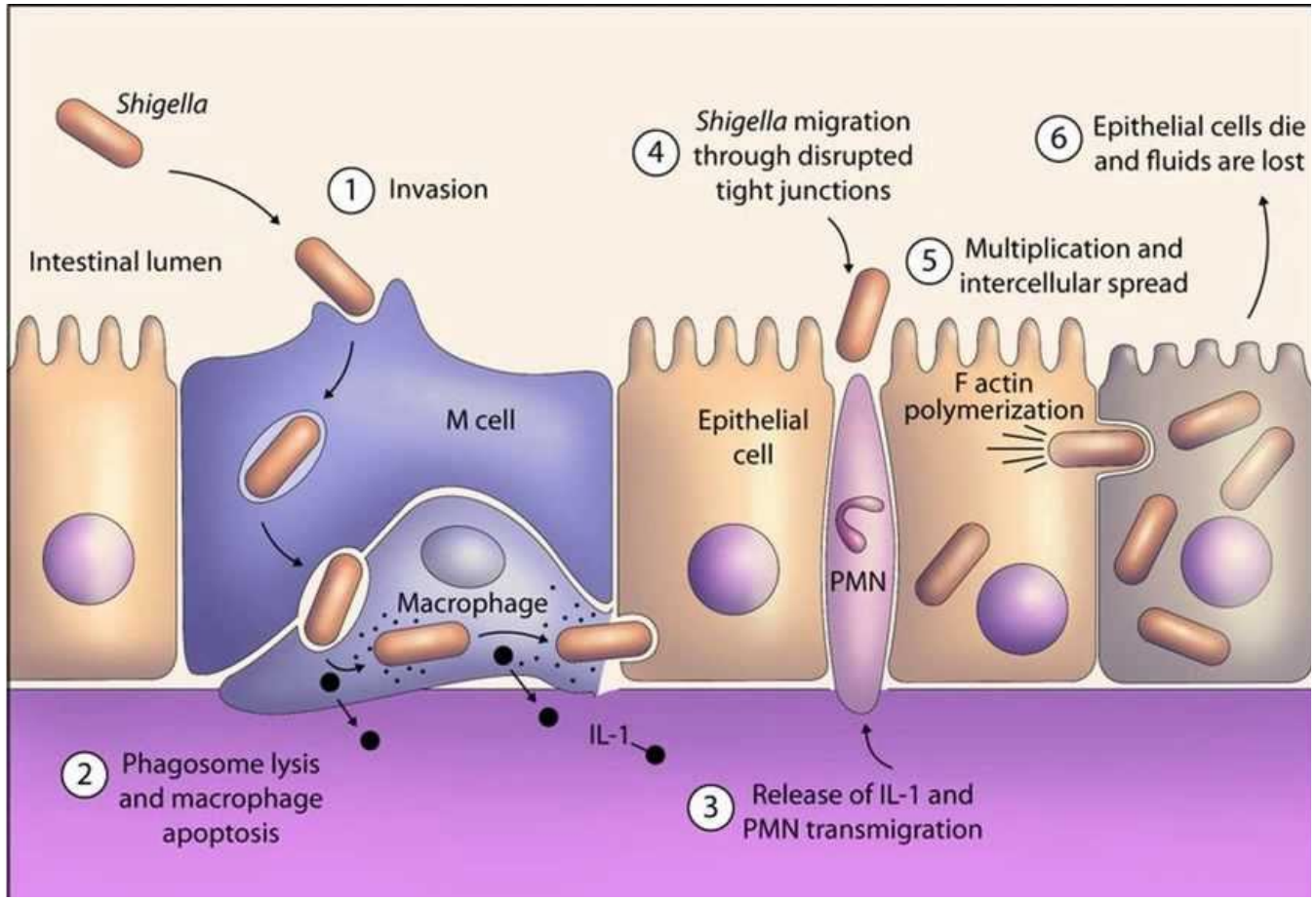
- **Shigellae** cause disease by invading and replicating in cells lining the **colon.** Structural gene proteins mediate the adherence of the organisms to the cells, as well as their invasion, intracellular replication, and cell-to-cell spread .

- *S. dysenteriae* strains produce an exotoxin, **Shiga toxin.** Similar to Shiga toxin produced by STEC

- The A subunit in the toxin cleaves the 28S rRNA in the 60S ribosomal subunit, thereby preventing the binding of aminoacyl-transfer RNA and **disrupting protein synthesis**.

- The primary manifestation of toxin activity is **damage to the intestinal epithelium**; however, in a small subset of patients, the Shiga toxin can mediate **damage to the glomerular endothelial cells, resulting in renal failure (HUS**).

Shigella passes the epithelial cell (EC) barrier by **transcytosis through M cells** and encounters resident macrophages. The bacteria **evade degradation in macrophages by inducing an apoptosis-like cell death**, which is accompanied by proinflammatory signaling. Free bacteria **invade the EC** from the basolateral side, **move into the cytoplasm by actin polymerization, and spread to adjacent cells**. **Proinflammatory signaling** by macrophages and EC further activates the innate immune response and attracts PMN. The influx of PMN **disintegrates the EC lining**, which initially exacerbates the infection and tissue destruction by facilitating the invasion of more bacteria. Ultimately, PMN phagocytose and kill Shigella, thus contributing to the resolution of the infection.



Shigella / Epidemiology and diseases

- **Humans are the only reservoir** for *Shigella*.

- ***S. sonnei*** is responsible for almost 85% of U.S. infections, whereas ***S. flexneri*** predominates in developing countries. Epidemics of ***S. dysenteriae*** infections occur periodically, most recently in West Africa and Central America.

- **Shigellosis** (Shigella infection) (is primarily a pediatric disease, with 60% of all infections in children younger than 10 years.

- Shigellosis is **transmitted person to person by** the fecal-oral route. Because as few as 100 to 200 bacteria can establish disease, shigellosis spreads rapidly in communities where sanitary standards and the level of personal hygiene are low.

- **Shigellosis** is characterized by **abdominal cramps, diarrhea, fever,** and **bloody stools.** The clinical signs and symptoms of the disease appear 1 to 3 days after the bacteria are ingested.

- Infection is **generally self-limited**, although **antibiotic treatment** is recommended **to reduce the risk of secondary spread** to family members and other contacts.

Clinical Case 25-3 *Shigella* Infections in Day-Care Centers

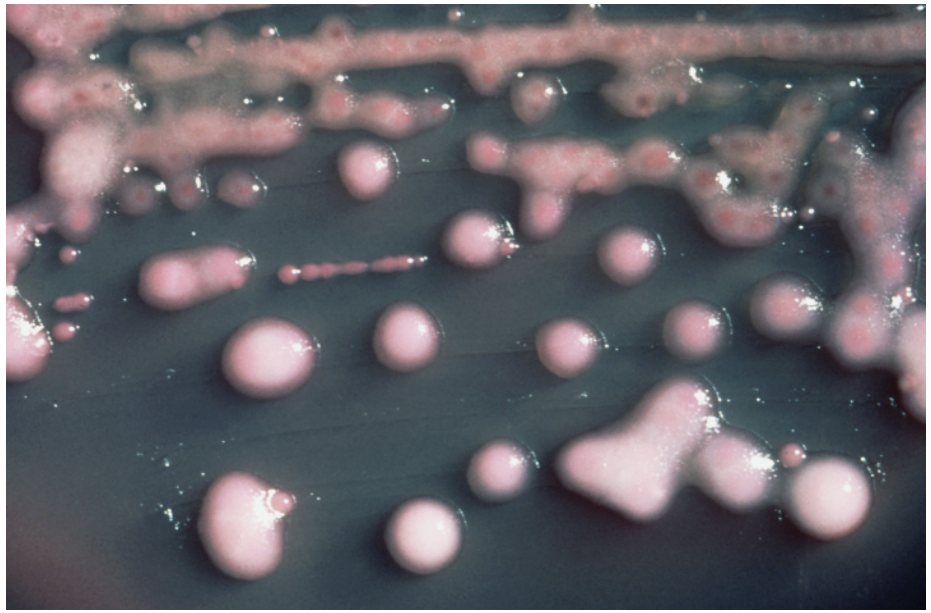
In 2005, three states reported outbreaks of multidrug-resistant *Shigella* infections in day-care centers. A total of 532 infections were reported in the Kansas City area, with the median age of patients 6 years old (Centers for Disease Control and Prevention: *MMWR Morb Mortal Wkly Rep* 55:1068–1071, 2006). The predominant pathogen was a multidrug-resistant strain of *Shigella sonnei,* with 89% of the isolates resistant to ampicillin and trimethoprim-sulfamethoxazole. Shigellosis spreads easily in day-care centers because of the increased risk of fecal contamination and the low infectious dose responsible for disease. Parents and teachers, as well as classmates, are at significant risk for disease.

Klebsiella

- *Klebsiella* species are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora.

- The most commonly isolated members of this genus are ***K. pneumoniae,*** which can cause community- or hospital-acquired primary **lobar pneumonia.** These bacteria **also cause wound and soft-tissue infections and UTIs**.

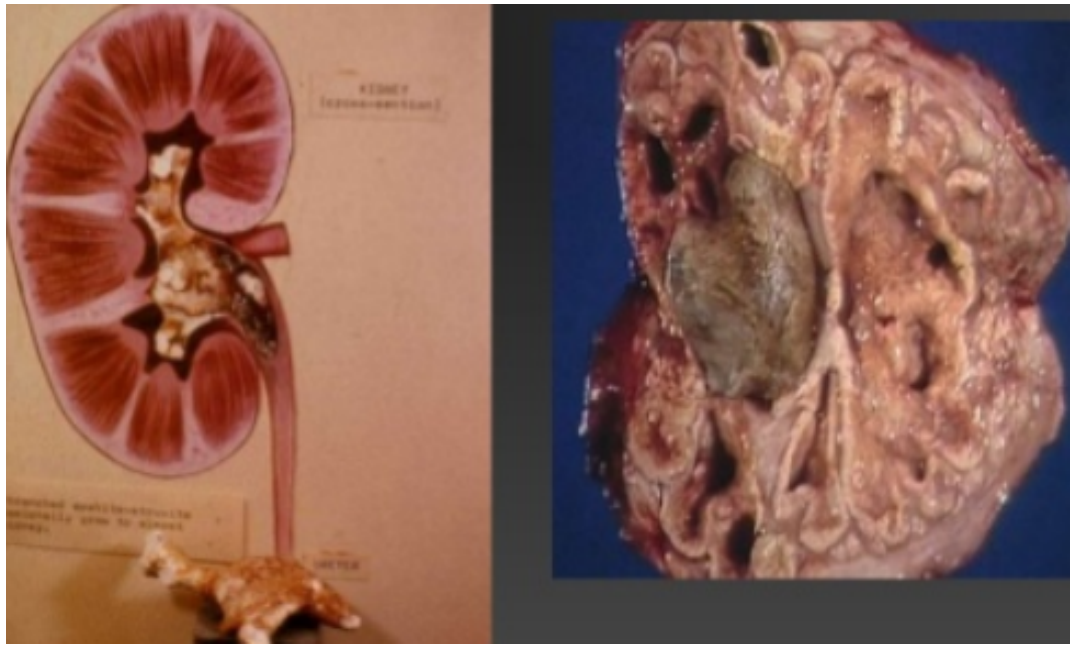
- The ability of *K. pneumoniae* to **colonize the hospital environment**, including carpeting, sinks, flowers, and various surfaces, as well as the skin of patients and hospital staff, has been identified as a major factor in the spread of **hospital-acquired infections**



Proteus

- ***P. mirabilis ,*** the most common member of this genus, primarily produces **infections of the urinary tract** .

- *P. mirabilis* produces large quantities of urease, which splits urea into carbon dioxide and ammonia. This process raises the urine pH, precipitating magnesium and calcium in the form of **struvite and apatite crystals**, respectively, and results in the formation of **renal (kidney) stones.** The increased alkalinity of the urine is also toxic to the uroepithelium



Yersinia

- The best-known human pathogen within the genus *Yersinia* is ***Y. pestis***

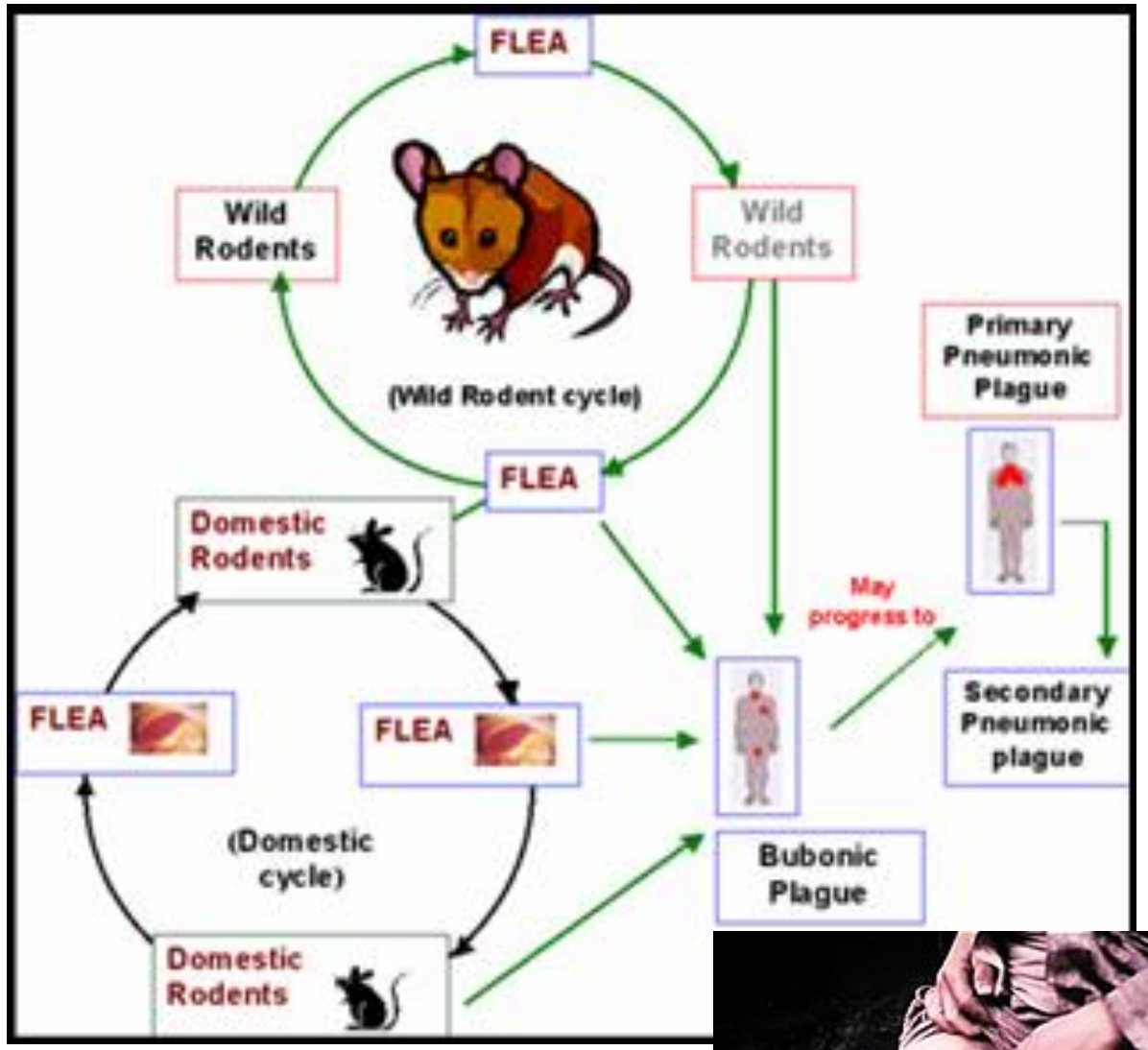
- All *Yersinia* infections are **zoonotic,** with humans the accidental hosts. There are two forms of *Y. pestis* infection: **urban plague,** for which rats are the natural reservoirs, and **sylvatic plague,** which causes infections in squirrels, rabbits, field rats, and domestic cats.

- **3 major pandemics that shaped history.**

Yersinia

- **Bubonic plague caused by *Y. pestis*** is characterized by an incubation period of no more than 7 days after a person has been bitten by an infected flea. Patients have a high fever and a painful **bubo** (inflammatory swelling of the lymph nodes) in the groin or axilla. Bacteremia develops rapidly if patients are not treated, and as many as 75% die.

- The patients are highly infectious; person-to-person spread occurs by aerosols in case of **pneumonic plague.**



The Justinian Plague began in 541 AD and was followed by frequent outbreaks over the next two hundred years that eventually killed over 25 million people and affected much of the Mediterranean basin–virtually all of the known world at that time.

The second pandemic, widely known as the “Black Death” or the Great Plague, originated in China in 1334 and spread along the great trade routes to Constantinople and then to Europe, where it claimed an estimated 60% of the European population, around 50-200 million lives.

The third pandemic, the Modern Plague, began in China in the 1860s and appeared in Hong Kong by 1894. Over the next 20 years, it spread to port cities around the world by rats on steamships. The pandemic caused approximately 10 million deaths


The evolution of bacteria
on a "mega-plate" Petri dish
HARVARD
MEDICAL SCHOOL

Further reading:

- Murray - Medical Microbiology 8th Edition
  Section 4: Bacteriology
  Chapter 25: ENTEROBACTERIACEAE