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Microbiology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

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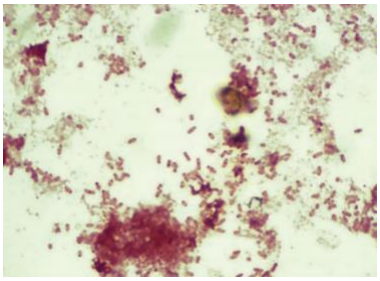
In this sheet we'll talk about different genera and species of gram-negative rods Enterobacteriaceae family and their properties, have fun..

General Properties:

1-They have **moderate** size.
2-They are **non-spore-forming**.
3-Most are **ubiquitous** (found everywhere)
4-Most are **facultative anaerobes**
5-They share a common antigen called: **enterobacterial common antigen.**



→Notice that they look under the microscope like any other gram-negative rods, with no special characteristics.

Classification according to Pathogenicity:

1-Part of the normal intestinal flora. (causes no infections)
2-*Always* associated with human disease when present in clinical specimens.
3-Members of the normal flora but can cause *opportunistic* infections. e.g., E. coli.
4-Normally commensal organisms but become pathogenic when they acquire virulence genes by transduction for example.
NOTE: we are not required to know the scientific classification (starting from domain, phylum, etc..) because it's always changing.

Virulence factors and protection mechanisms:

1-Endotoxin: a toxin whose activity depends on the lipid A component of LPS, released at cell lysis. Remember that these are **gram-negative** bacteria, so they have an outer membrane, and an inner membrane, separated by periplasmic space. This outer membrane has a lipopolysaccharide (LPS) layer.

2-Flagella (aka. H antigen): Provide *motility*.

3-Capsule (aka. K antigen): Prevent phagocytosis. However, anticapsular antibodies *diminish* the capsule role.

4-Pili

5-Type III Secretion Systems: secrete exotoxins.

6-Antigenic phase variation: These bacteria can **hide** antigens which can be recognized by the immune system (O antigen for example), to evade it.

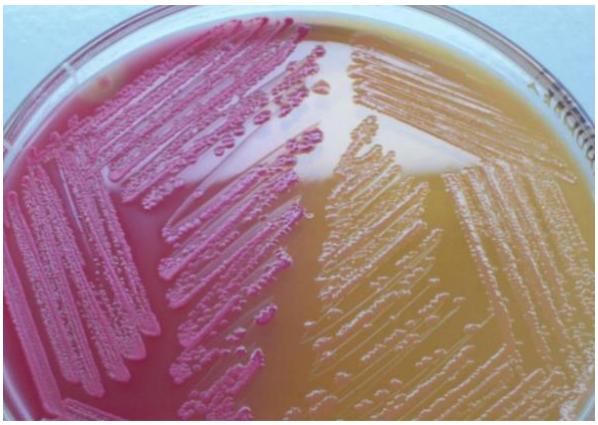
7-Iron sequestration: stealing iron from our cells, either by **producing iron-chelating** compounds (enterobactin, aerobactin) or by **RBCs lysis**)

8-Serum Killing and Antimicrobial Resistance.

NOTE: Antigens help in serotyping and designation of different species, as we'll see later

NOTE: Not all strains have all types of virulence factors, so some strains are motile while others are not, and some strains are capsulated while others or not, and so on.

*Enterobacteriaceae are **not** fastidious..*
*They can be grown on almost any nutrient agar. However, we can use **MacConkey's agar** containing bile acid to ensure that these colonies are gram negatives. Adding a natural red dye can help us differentiate between **Lactose-fermenters** and **non-fermenters.** If we want to further differentiate between species, other biochemical tests and molecular techniques may be used.*



Antibiotic resistance: Gram-negative bacteria are naturally resistant to many β-lactams thanks to its outer membrane and drug efflux mechanism. However, using broad-spectrum antibiotics or when they are not needed, makes it even worse that some species become Multi-drug resistant (MDR) and can even share this resistance with their bacteria friends.

Now we'll take the most important pathogens of this family, one by one.

1)Escherichia coli:

▪ **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 **diarrheal disease** and the most frequent cause of **bloodstream infection** as well as **urinary tract infections (UTIs)** among Gram-negative bacteria.

They are divided into 3 groups:

1)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.

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

3)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.

The Diarrhoeagenic strains of E. coli are subdivided into 5 groups according to pathogenesis. Two of these groups will be in the focus of this course:

1)ETEC, Enterotoxigenic E. coli **2) STEC, Shiga toxin-producing E. coli.**
3)EPEC, Enteropathogenic E. coli. 4)EAEC, Enteroaggregative E. coli
5) EHEC, Enterohemorrhagic E. coli

Traveler's diarrhea:
When you travel to a place where the climate or sanitary practices are different from yours at home, you have an increased risk of developing diarrhea (by whatever pathogen). Usually recovery happens within days with no need for treatment.

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1)Enterotoxigenic E. coli (ETEC):

A) One of the most common causes of **bacterial diarrheal disease in developing countries** and **30% of traveler's diarrhea.**

B) Because the inoculum for disease is **high**, infections are primarily acquired through consumption of fecally contaminated food or water. **Person-to-person spread does not occur.**

C) **Secretory diarrhea** caused by ETEC develops after a 1-2 day incubation period and persists for an average of 3-5 days.

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

E) **ETEC** produce *two* classes of enterotoxins:

1) Heat-stable toxins (ST): leads to *increase* in cyclic guanosine monophosphate (cGMP) and subsequent hypersecretion of fluids into the lumen. It can also *inhibit* the fluid absorption (by damaging intestinal epithelium).

2) Heat-labile toxins (LT): leads to increase in cyclic adenosine monophosphate (cAMP) levels, resulting in enhanced secretion of chloride and decreased absorption of sodium and chloride. **Less sodium absorbed = Less water absorbed**

***Test your understanding**:*

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.

2)Shiga toxin–producing E. coli (STEC):

A) 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.**

B) Disease caused by STEC ranges from **mild** uncomplicated diarrhea to **hemorrhagic colitis** with **severe abdominal pain** and **bloody diarrhea** (where Shiga toxin reaches the colon and causes inflammation). Severe disease, like the one in 2006 outbreak, is more commonly associated with **STEC O157:H7 (so this strain has O antigen and Flagella).**

C) Complete resolution of symptoms typically occurs after **4 to 10** days in most untreated patients.

D) If the toxin reaches blood and travel to kidneys it can cause **Hemolytic uremic syndrome (HUS)**, a disorder characterized by acute renal failure. *Thrombocytopenia, and microangiopathic hemolytic anemia* can result from STEC infection as well.

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

In 2006, *E. coli* O157 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.

*Things that we need to remember about STEC are underlined.

Gastroenteritis is a general term describing inflammation of GI tract.

Extraintestinal infections of E. coli:

A) **Urinary Tract Infection:** E. coli, especially strains that have **fimbriae** and other adhesion molecules (*to prevent flushing out with urine*), are the most common cause of UTIs. They originate in the colon, contaminate the urethra, ascend into the bladder, and may migrate to the kidney or prostate.

B) Almost every woman suffers from a bladder infection at some point in her life (*E. coli in 80% of UTI cases*). Men are also 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).

C) E. coli and group B streptococci cause the majority of CNS infections in infants younger than 1 month e.g. **neonatal meningitis**. Newborns get these bacteria while passing through vaginal canal.

D) **Septicemia**: caused by gram-negative rods, such as E. coli, most commonly originates from **infections in the urinary or GI tract** (e.g., intestinal leakage leading to an intraabdominal infection) with high mortality in patients.

2) Salmonella

Most salmonella types are found as **normal commensals** in **poultry** without causing any infections, but when humans ingest, for example, *undercooked chicken, eggs, dairy products, and foods* prepared on contaminated work surfaces they can become infected. In this case **large** inoculum (e.g., 106 to 108 bacteria) is required for symptomatic disease.

But some serotypes like *Salmonella Typhi and Salmonella Paratyphi* are highly adapted to humans and do not cause disease in nonhuman hosts, **person-to-person spread occurs when food or water contaminated by infected food handlers is ingested**. In this case the infectious dose for Salmonella Typhi infections is **low**.

After *ingestion and passage* through the stomach→ salmonellae *attach* to the mucosa of the small intestine → *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 but the inflammatory response *confines the infection to the GI tract* → It mediates the release of *prostaglandins*, and stimulates cAMP and active fluid secretion causing **diarrhea**

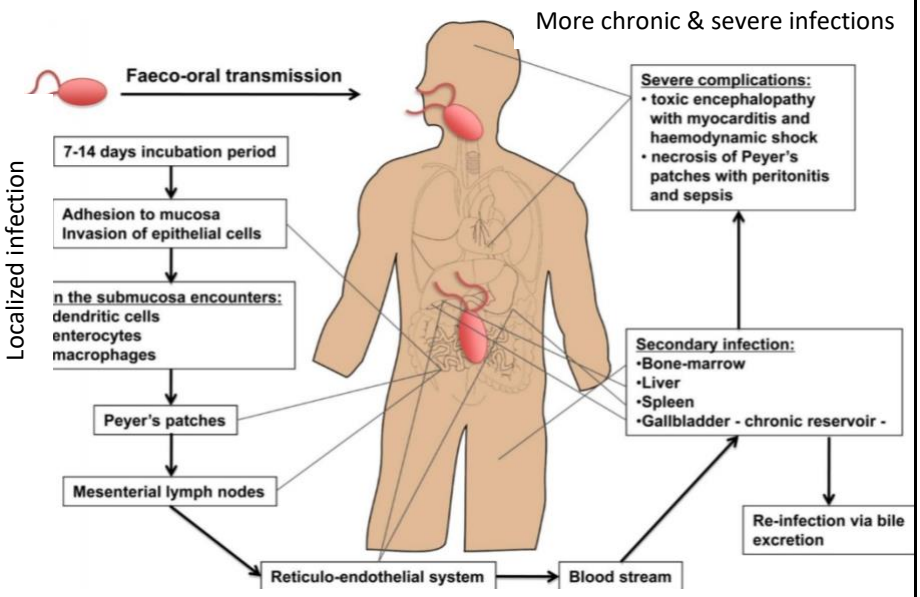
❖ Diseases caused by salmonella

*Virulence depends on pathogenicity island on the bacterial chromosome encoding for **toxins, attachment proteins and immune evasion** mechanisms.*

▪ **Gastroenteritis**: 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**, but infections with Salmonella Typhi, Salmonella Paratyphi more commonly lead to a bacteremic phase.

▪ **Typhoid fever**: a *febrile* illness caused by S. Typhi. A *milder* form of this disease, called paratyphoid fever, 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. The infection in this case is **not contained** in the intestine and instead moves into the *circulation*, reaching the liver, spleen, and bone marrow where the bacteria replicate. 10-14 days after ingestion of the bacteria, patients experience **gradually increasing fever**, with nonspecific complaints of headache, myalgias, malaise, anorexia, it can also lead to **meningitis**). *Systemic complications → must start giving antibiotics.*



》 The strains of Salmonella responsible for causing typhoid and paratyphoid can cause asymptomatic colonization. Which makes it easier to transmit to other individuals. The 1st person to be identified as an asymptomatic carrier of these pathogens is Mary Mallon, also known as "typhoid Mary". She infected 51 people while working as a cook. It also seems that she was unhygienic.

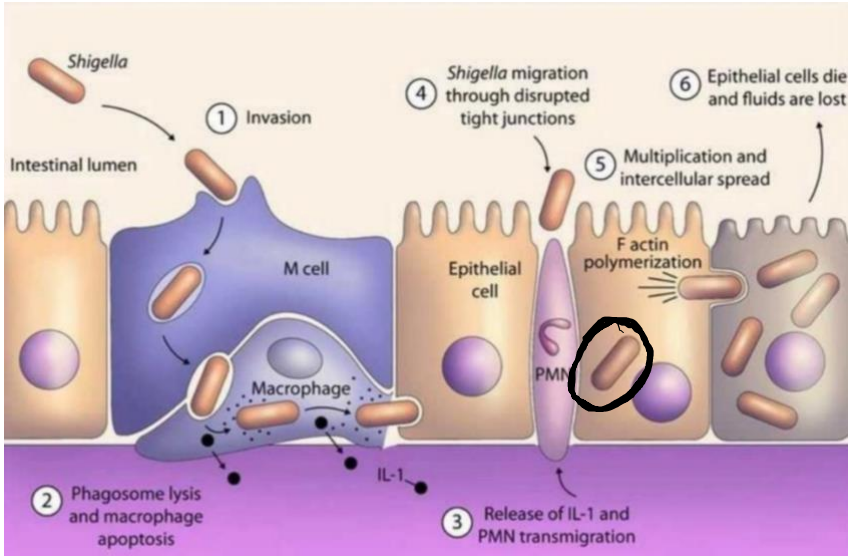
• Read the following clinical case and just understand the concept

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.

3) Shigella

• Includes *Shigella dysenteriae, S. flexneri, S. boydii, and S. sonnei*. But, analysis of DNA has determined that these 4 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.



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.

Diseases caused by shigella

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).**

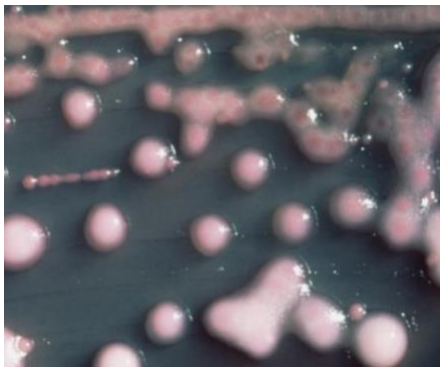
Shigellosis (Shigella infection) (is primarily a **pediatric** disease, with 60% of all infections in children younger than 10 years)

⇒ It 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** stool*s. 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

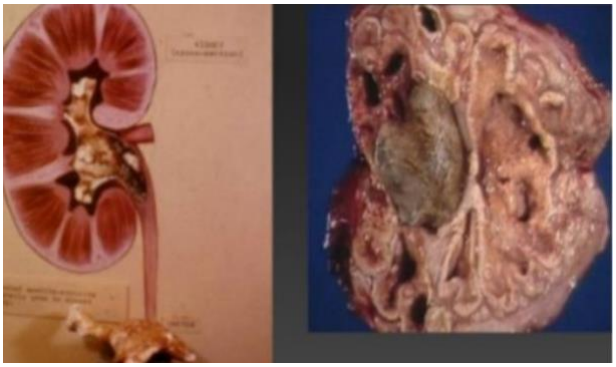
In 2005, three states reported outbreaks of multidrug-resistant *Shigella* infections in day-care centers. A total of 532 infections were reported in the Kansas City area, with the median age of patients 6 years old (Centers for Disease Control and Prevention: *MMWR Morb Mortal Wkly Rep* 55:1068–1071, 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.

4) Klebsiella



▪ Routinely found in the *human nose, mouth, and GIT as normal flora.*
▪ The most commonly isolated members of this genus are **K. pneumoniae**, which can cause **community- or hospital-acquired primary lobar pneumonia**, **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*.

5) Proteus



P. mirabilis, the most common member of this genus, primarily produces **infections of the urinary tract.** It 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 **large renal (kidney) stones**. The increased alkalinity of the urine is also toxic to the uroepithelium.

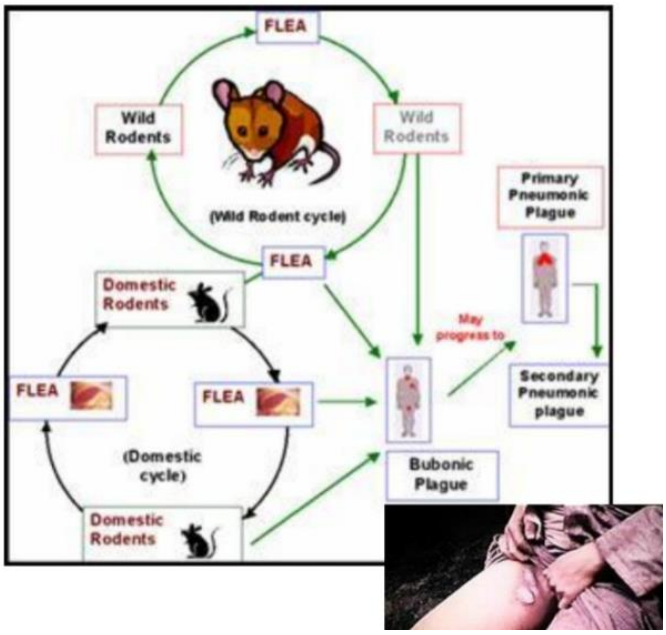
Most common cause of UTI is enteriobacteriacae : #1 is E.coli. Followed by, kleibsella and proteus

6) Yersinia

- **Y. pestis** is the best-known human pathogen within the genus Yersinia.
- 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	Sylvatic plague, which causes infections in squirrels, rabbits, field rats, and domestic cats



However, in humans, it causes **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 and sepsis** develop rapidly if patients are not treated, and as many as 75% die. Can also reach the lungs causing **pneumonic plague** which makes the patients highly infectious and person-to-person spread occurs by aerosols in this case. Check the following picture of how the pathogen can be transmitted to humans.

the 3 pandemics cause by Y. Pestis

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.

Doctors at that time wore this clothing to protect themselves from diseases. You can use this to remind you of the two plagues that effect humans caused by Y. Pestis:
Bubonic plague: so protect the body from flea bites
Pneumonic plague: protect the RT from entry of airborne pathogens
*But remember at that time they believed in the miasma (bad air) theory. So they didn't know about the pathogens then.

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
Number of deaths has decreased because of improvement of sanitation and more knowledge about the diseases.

GOOD LUCK