Zoonotic Viruses

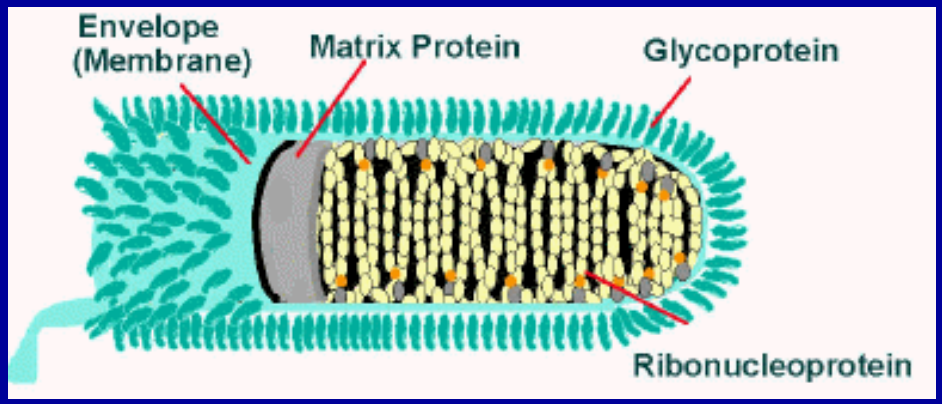
- Zoonoses are diseases of vertebrate animals that can be transmitted to man: either directly or indirectly through an insect vector.
- When an insect vector is involved, the disease is also known as an arboviral disease.
- However, not all arboviral diseases are zoonosis: where the transmission cycle takes place exclusively between insect vector and human e.g. dengue and urban yellow fever.
- Examples of viral zoonoses that can be transmitted to man directly include rabies, hantaviruses, lassa and ebola fevers.

Rabies Virus

- member of the Lyassavirus of the Rhabdoviridae
- ssRNA enveloped virus, characteristic bullet-shaped appearance with 6-7 nm spike projections.
- virion 130-240nm * 80nm
- -ve stranded RNA codes for 5 proteins; G, M, N, L, S
- Exceedingly wide range of hosts
- There are 5 other members of Lyassavirus : Mokola, Lagosbat, Duvenhage, EBL-1, and EBL-2
- Duvenhage and EBL-2 have been associated with human rabies.

Rabies Virus

Rabies virus particles



Structure of rabies virus (Source: CDC)

Epidemiology

- Rabies is a zoonosis which is prevalent in wildlife. The main animals involved differs from continent to continent.

Europe	fox, bats
Middle East	wolf, dog
Asia	dog
Africa	dog, mongoose, antelope
N America	foxes, skunks, raccoons, insectivorous bats
S America	dog, vampire bats

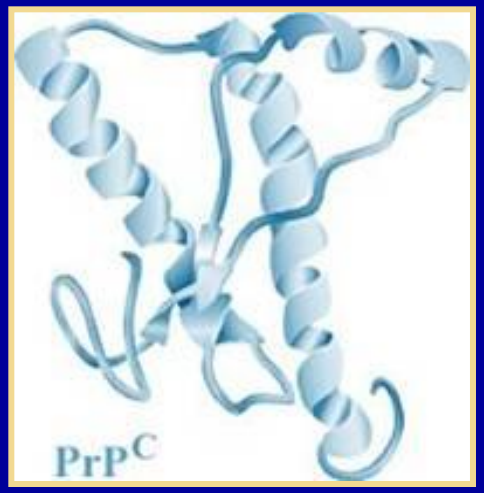
Spongiform Encephalopathies and Prions

Prion

- Smaller than smallest known virus
- Not yet completely characterized
- Most widely accepted theory
  - Prion = Proteinaceous infectious particle
- Normal Protein
  - PrP^{C} (C for cellular)
  - Glycoprotein normally found at cell surface inserted in plasma membrane

Normal protein

- Secondary structure dominated by alpha helices
- Easily soluble
- Easily digested by proteases
- Encoded by PRNP gene (in humans)
  - Located on human chromosome 20

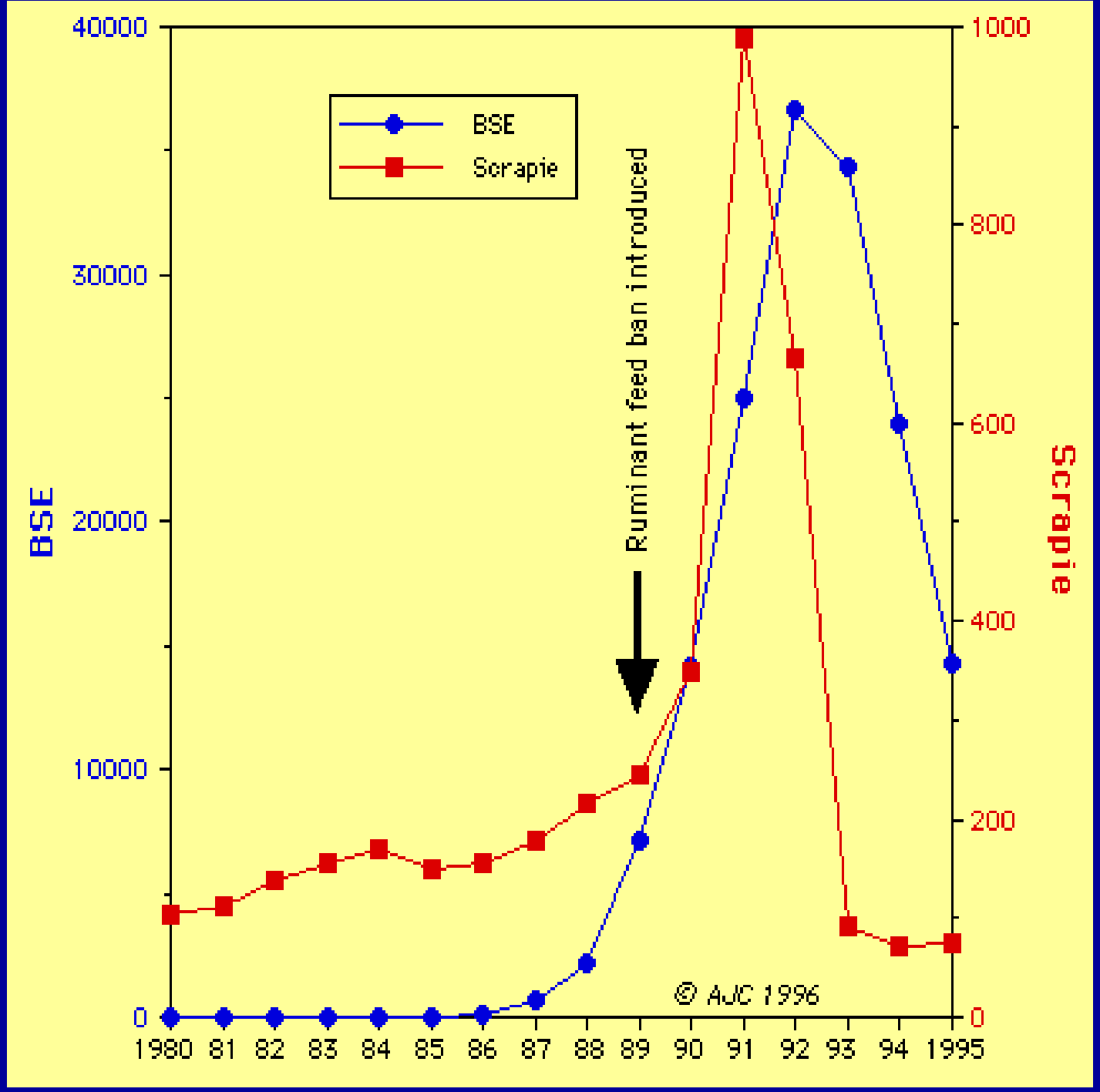


Abnormal Protein

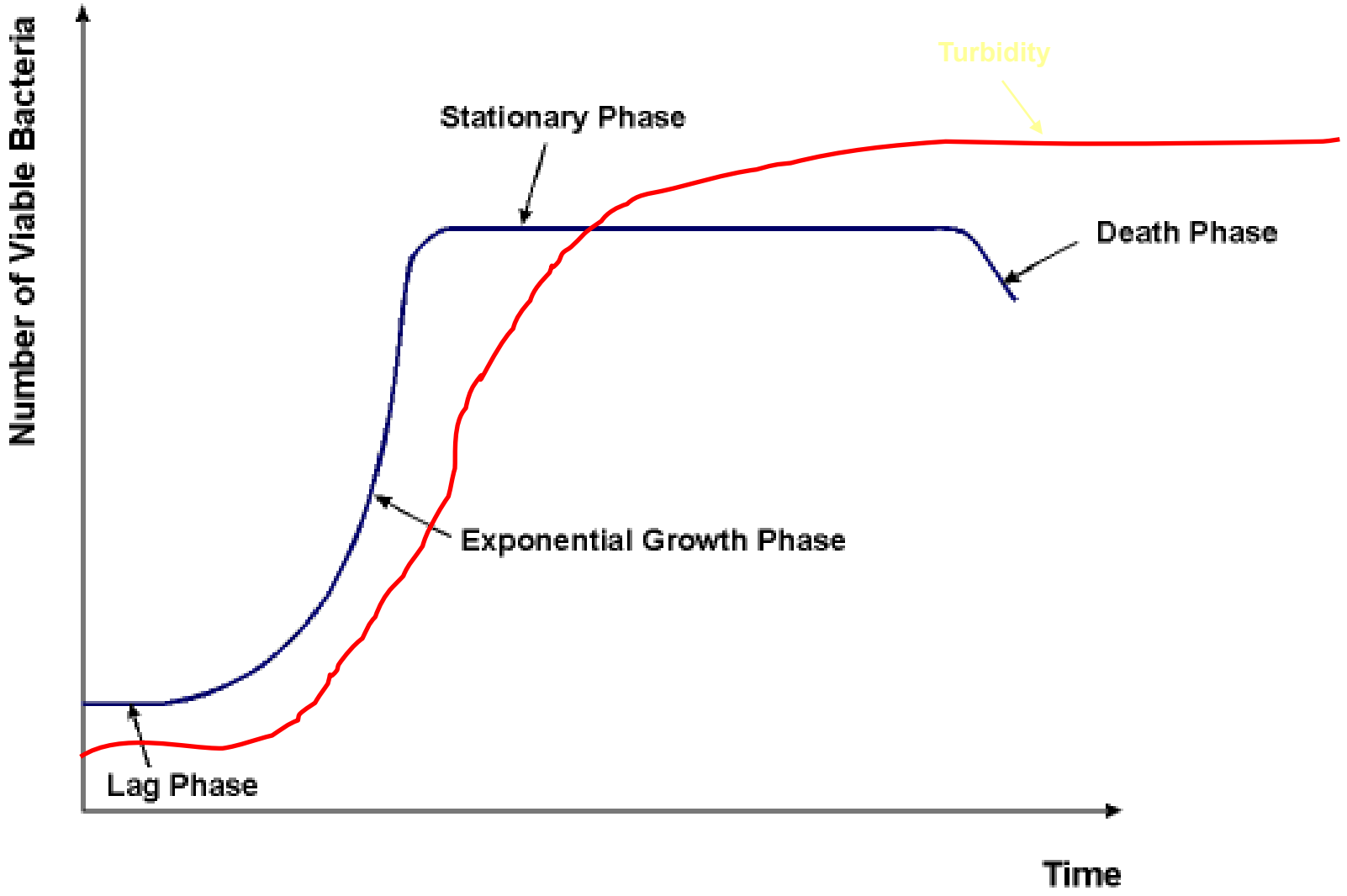
- Insoluble in all but strongest solvents
- Highly resistant to digestion by proteases
  - Survives in tissues post-mortem
- Extremely resistant
  - Heat, normal sterilization processes, sunlight
- No detectable immune response

spongiform encephalopathies of humans and other animals

- humans
  - Kuru (Fore people in New Guinea)
  - Creutzfeldt-Jakob disease (CJD)
  - Gerstmann-Straussler-Scheinker (GSS)
  - fatal familial insomnia
  - new variant CJD
- Animals
  - Scrapie
  - exotic ungulate encephalopathy
  - bovine spongiform encephalopathy (BSE)
  - transmissible mink encephalopathy
  - feline spongiform encephalopathy
  - chronic wasting disease (CWD)


BSE
Scrapie
Ruminant feed ban introduced
© AJC 1996
40000
30000
20000
10000
0
1000
800
600
400
200
0
1980 81 82 83 84 85 86 87 88 89 90 91 92 93 94 1995

Bacterial Pathogens


Number of Viable Bacteria
Turbidity
Stationary Phase
Death Phase
Exponential Growth Phase
Lag Phase
Time

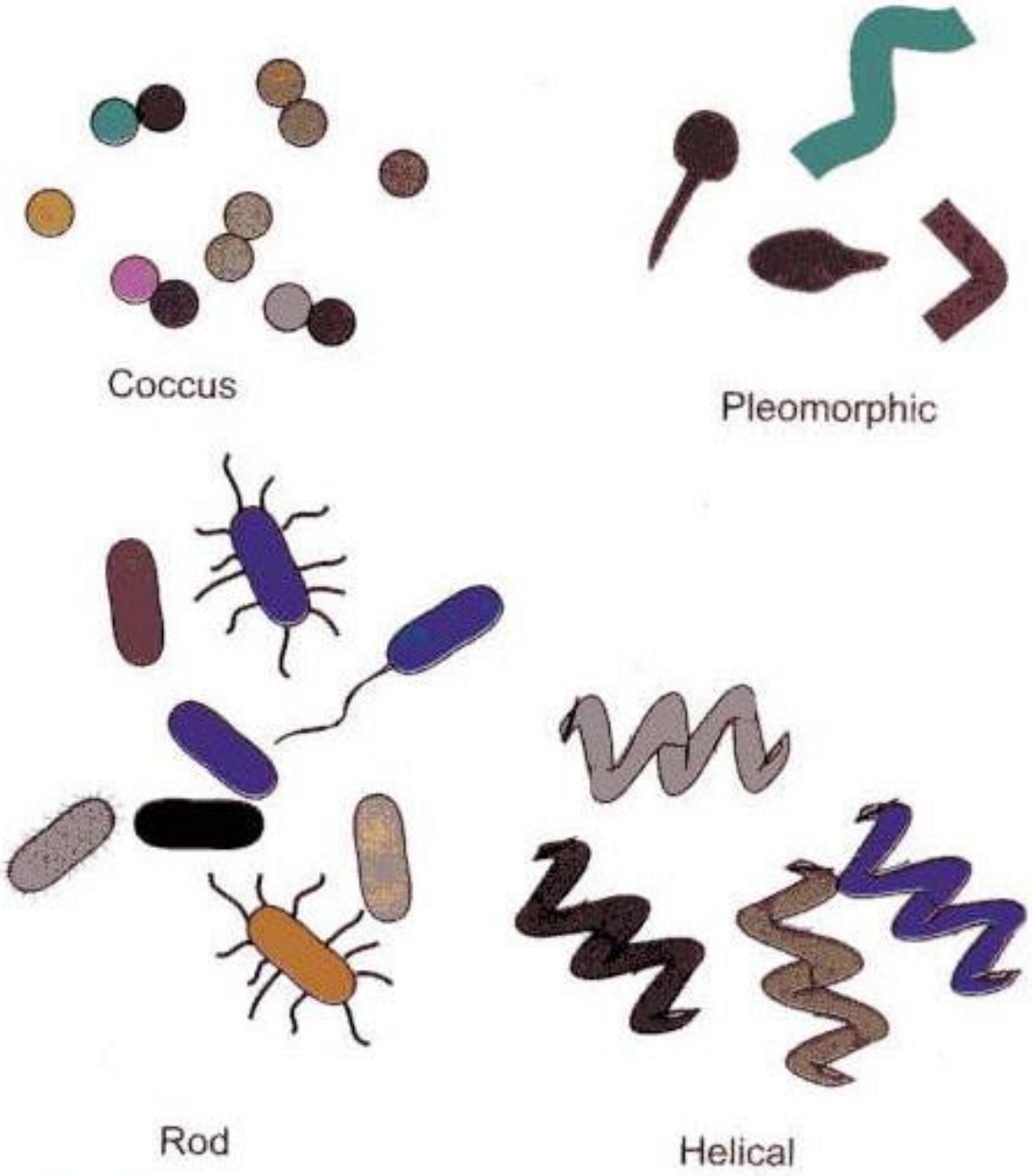


FIGURE 2.7 Typical shapes of representative bacteria.

Pathogenic Bacteria

Pathogenic bacteria possess virulence properties in the form of structures or chemical constituents that contribute to pathophysiology

- Endotoxins
- Exotoxins

Pili: for attachment and effacement to cells and tissues

Invasins: to invade cells

Some bacteria make spores:

- highly to physical and chemical agents and
- very persistent in the environment

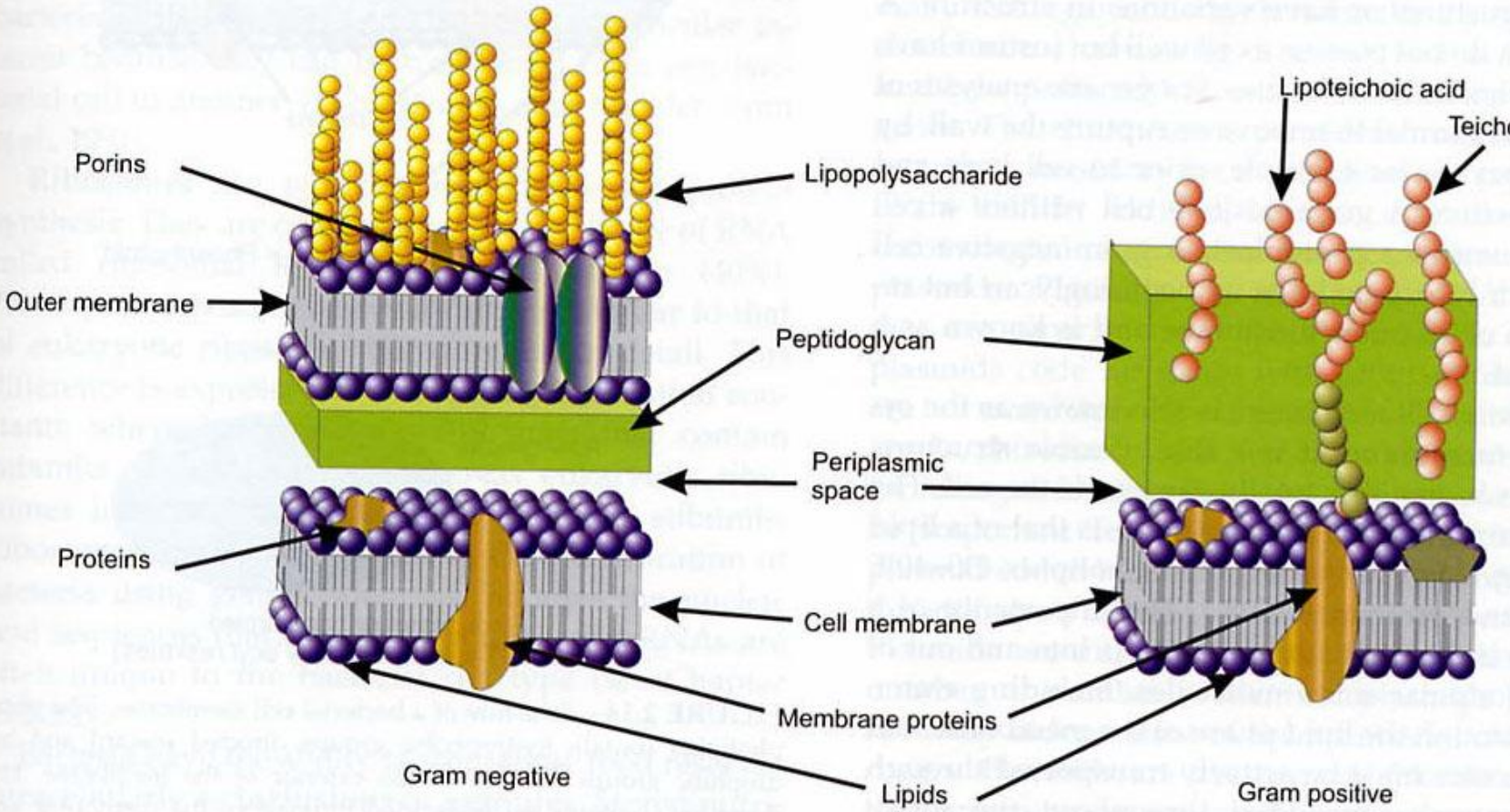
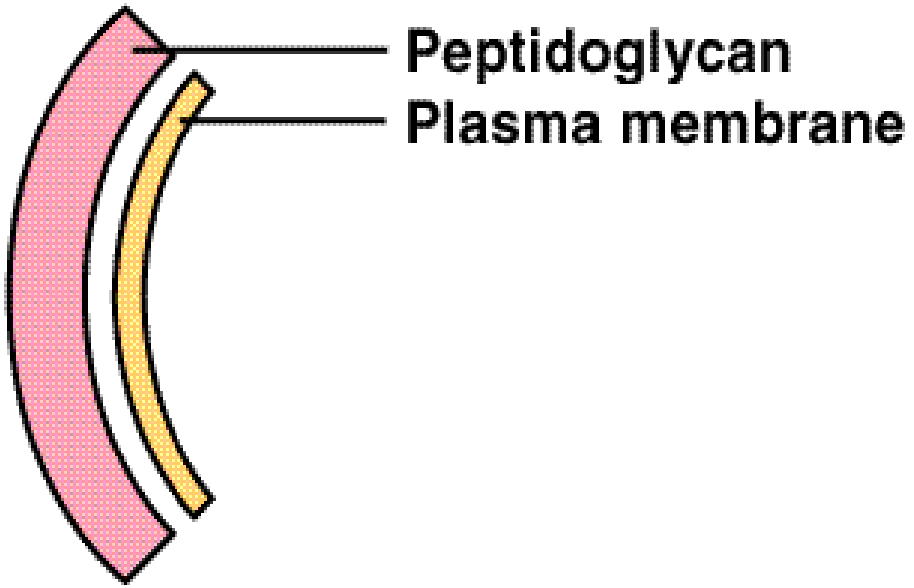


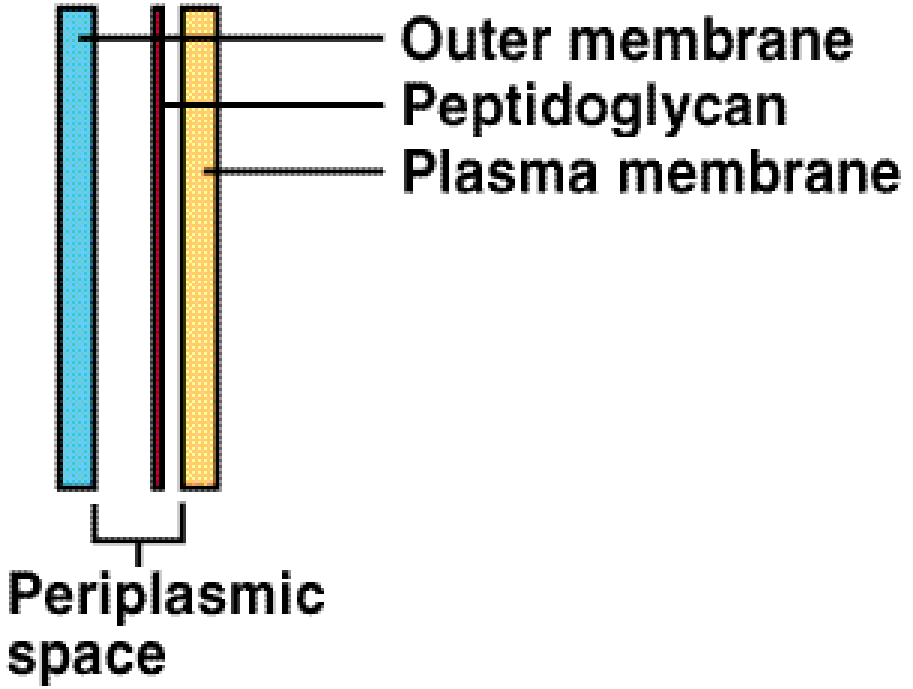
FIGURE 2.12 Comparison of gram-positive and gram-negative bacterial cell walls.

Bacterial Cell Wall

The gram-positive cell wall



The gram-negative cell wall



Peptidoglycan

- Responsible for rigidity of the cell
- 5-10% of wall mass in G-
- 60-70% of wall mass in G+
- Not a permeability barrier
- Site of action of penicillin- effects G+ much more than G-
- Archebacteria have modified version

Outer envelope

- Unique to G- bacteria
- Can contain enzymes or enzyme like materials
- Can act as permeability barrier but less so than plasma membrane
- Contains endotoxins, antigens, a big mix of different materials

Capsule

- Outside the cell wall- usually present only when cell has adequate carbon
- Composed of polysaccharide- glycocalyx
- External storage for carbon
- Protect from phagocytosis
- Glue

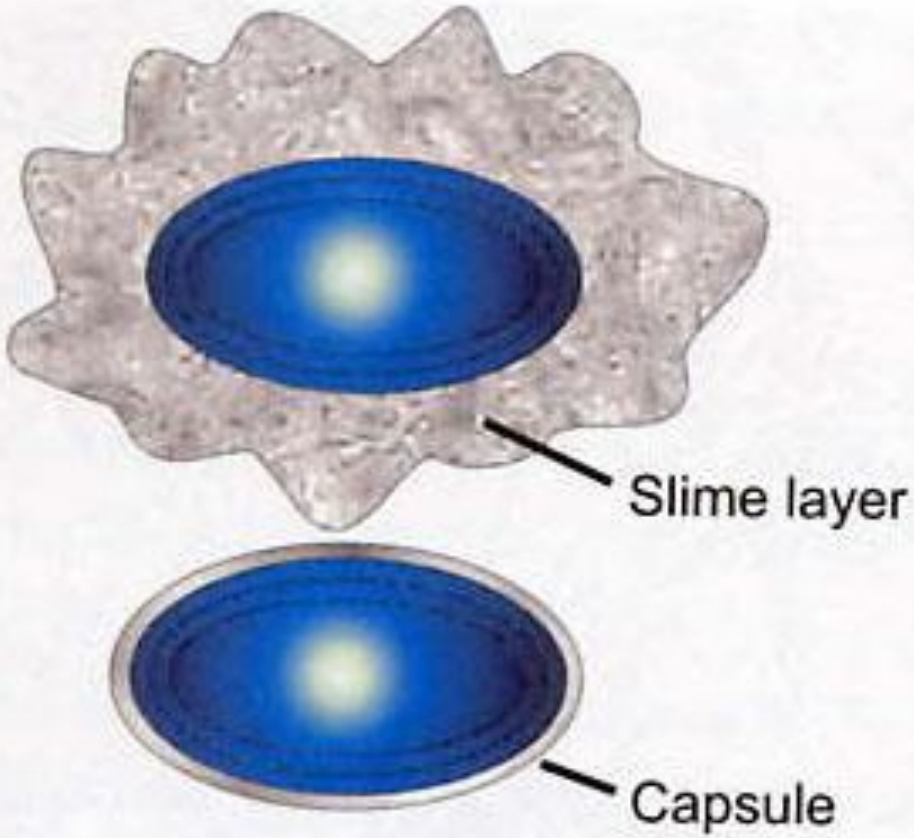


FIGURE 2.11 Two different types of glycocalyces: (1) slime layer; and (2) capsule.

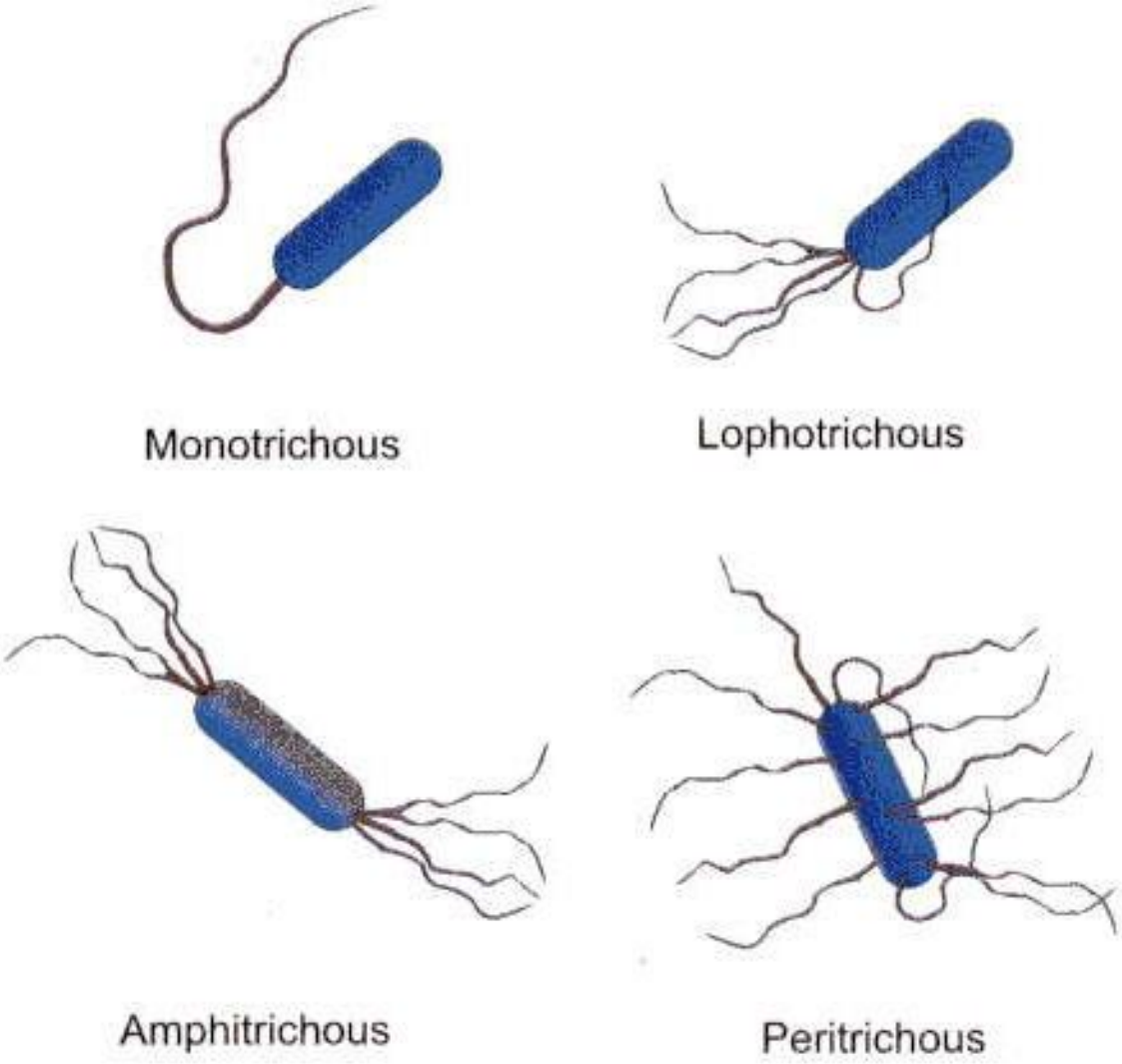
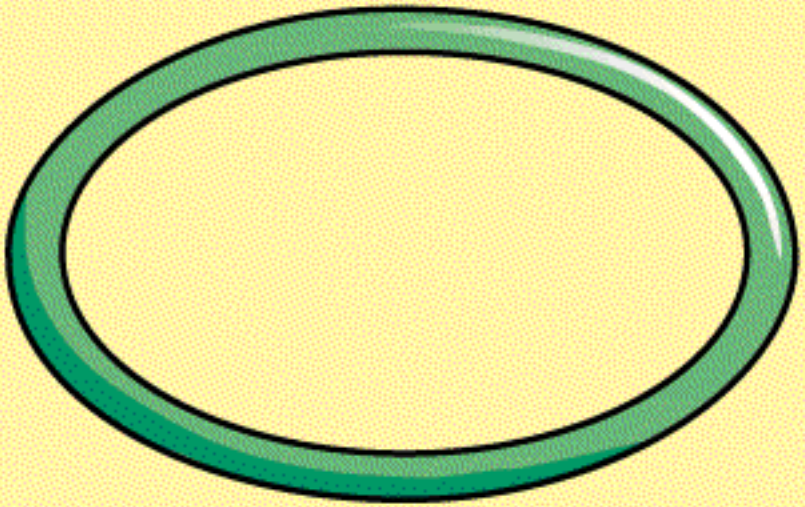


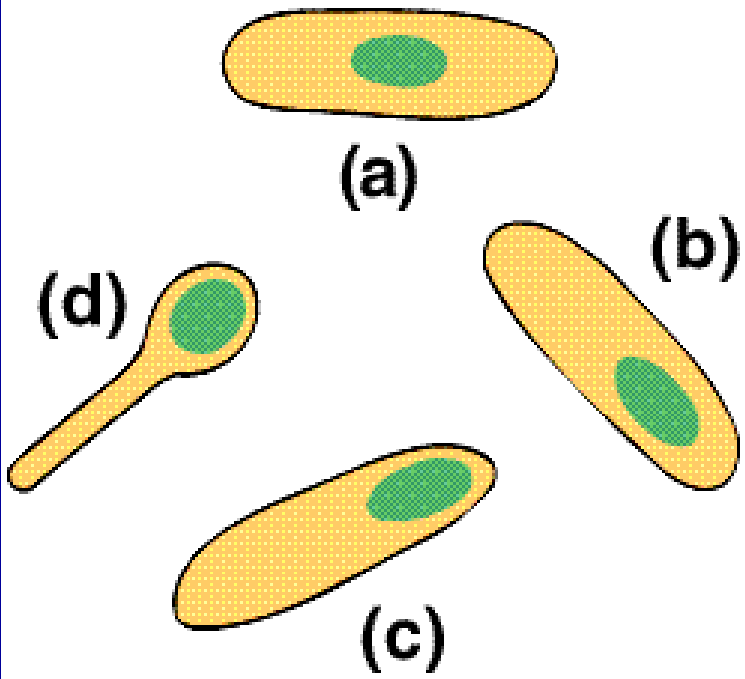
FIGURE 2.10 Arrangement of flagella extending from the cell envelope.

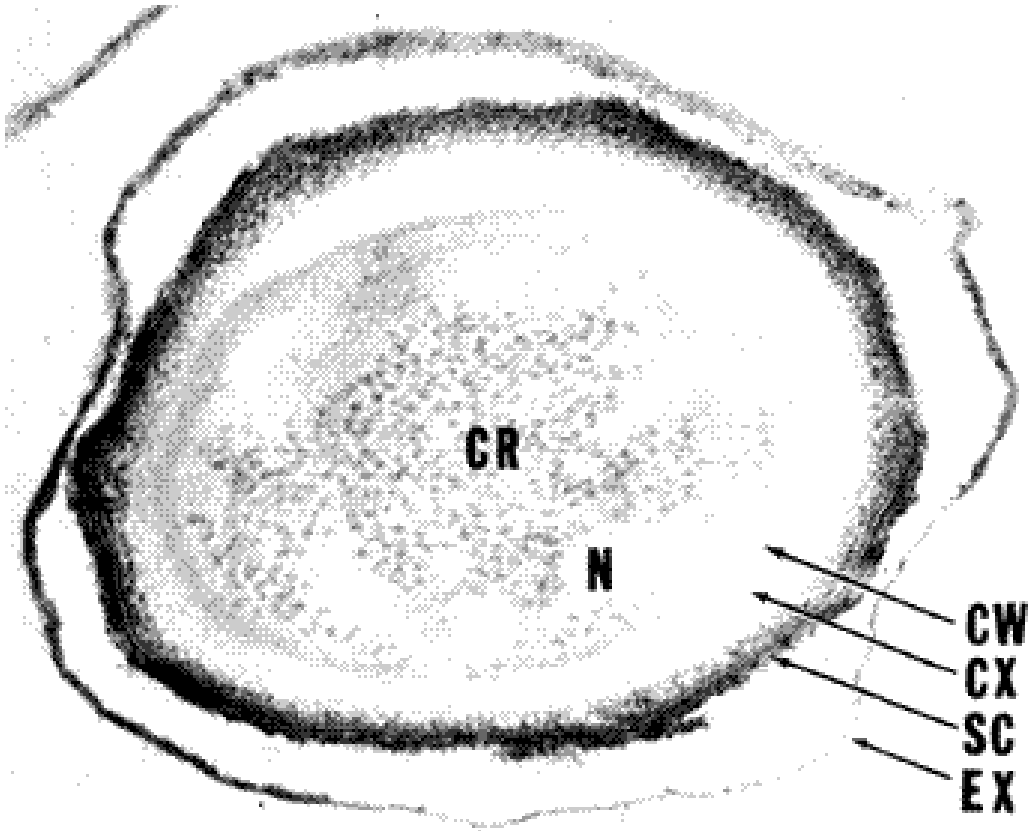


Open circle

Supercoiled

The Bacterial Endospore





American Society for Microbiology

Endospores

- Survival not reproduction
  - heat, drying, radiation, pH
- Turned on by running out of nutrients
- May be important in viable but not culturable phenomenon
- Great economic cost
- Major issue in food safety

START infoporn

The Bug Wars

In the battle of bad bacteria vs. antibiotics, the drugs usually lose.

Infectious diseases give us a stunning demonstration of evolution in action: The fittest bacteria – the ones that survive an antibiotic onslaught – transfer their resistance to new generations and across species. Their ability to fight back usually strengthens with each mutation, allowing them to thwart even the most intelligently designed drugs. Over the past 63 years, deadly bugs like *Staphylococcus aureus, Streptococcus pneumoniae,* and *Escherichia coli* have evolved to withstand medicines like penicillin, tetracycline, and ciprofloxacin. So scientists are now planning a flank attack – precisely targeted drug-delivery systems and bacteria-eating nanorobots. But if history repeats itself, the bugs will ultimately win. **– Patrick Di Justo**

BEHIND ENEMY LINES: A LOOK AT RESISTANCE TACTICS

Genetic mutations enable bacteria to adapt to new threats. Here are three ways they evolve to combat antimicrobial agents.

CAMOUFLAGE
A bacterium's protein receptors morph so the antibiotic can't lock into them. (Staph used this method to evade the penicillin family.)

ROADBLOCKS
The cell membrane changes to keep the antibiotic out. (Bacteria like staph and strep fended off tetracyclines this way.)

DISARMAMENT
A bacterium produces enzymes that turn off the active part of the antibiotic. (This is how *E. coli* trumped cephalosporins.)

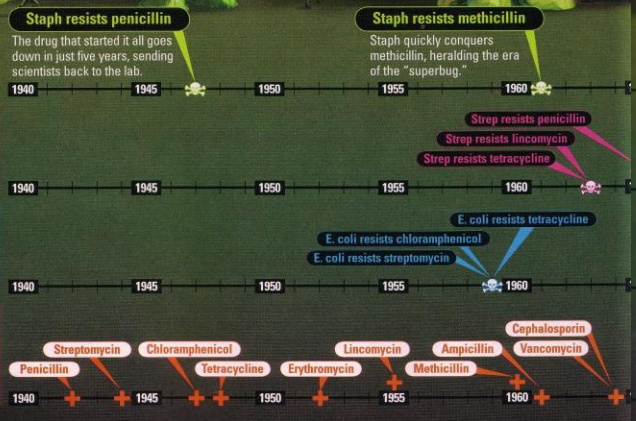
HOW FAST BACTERIA EVOLVE TO THWART DRUGS

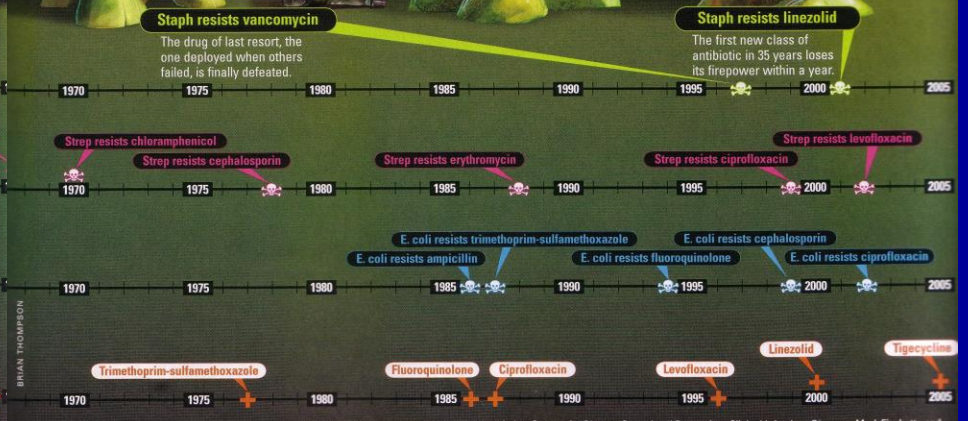
Staphylococcus aureus
S. aureus causes everything from skin infections to toxic shock syndrome. More than half of all staph infections found in intensive care units today can be linked to a drug-resistant strain.

Streptococcus pneumoniae
Besides the much-loathed strep throat and the much-feared flesh-eating bacteria, strains of *Streptococcus* cause over 125,000 cases of pneumonia a year that require hospitalization.

Escherichia coli
Dangerous forms of *E. coli* cause all sorts of maladies, from GI distress to meningitis. In June 2005, the FDA approved tigecycline, a new type of antibiotic designed to fight resistant *E. coli.*

Antibiotics
More than 110 million antibiotic prescriptions are written annually in the US. The Centers for Disease Control and Prevention discourages the use of antibiotics to treat viral illnesses like the flu. The drugs are ineffective against viruses.





BRIAN THOMPSON

Sources: Alliance for the Prudent Use of Antibiotics; Centers for Disease Control and Prevention; *Clinical Infectious Diseases*; Mark Fischetta and Elinor Levy, *The New Killer Diseases*; Jeffrey Fisher, *The Plague Makers*; Stuart Levy, *The Antibiotic Paradox*; National Library of Medicine

Bacterial Taxonomy

- Very little known about bacterial diversity, except in reference to illness
- Early attempts at classifying bacteria were based morphological, biochemical, and serologic (phenotypic) properties
- More recent classification methods also rely on genetic (and advanced phenotypic) methods
  - e.g. DNA-DNA hybridization studies, rRNA homology, etc.
  - Chemotaxonomy- FAA, WCPA, Cell wall composition, etc.
- Polyphasic taxonomy approach results in fluidity at genus level and below

Bacterial Phyla

- **Actinobacteria**
- Aquificae
- **Bacteroidetes**
- **Chlamydiae**
- Chlorobi
- **Cyanobacteria**
- Deinococcus
- **Firmicutes**
- Fusobacteria
- **Proteobacteria**
- **Spirochaetes**
- Thermotogae

Important Genera

Actinobacteria
- *Corynebacteria*
- *Mycobacteria*
- *Bifidobacteria*

Bacteroidetes
- *Porphyromonas*

Chlamydiae
- *Chlamydia*

Fimicutes
- Bacilliales
  - *Bacillus*
  - *Listeria*
  - Staphylococcus
- Clostridiales
  - *Clostridia*
- Lactobacillales
  - *Enterococcus*
  - *Lactobacillus*
  - *Lactococcus*
  - *Streptococcus*
- Mollicutes
  - *Mycoplasma*

Important Genera

Proteobacteria

- Alphaproteobacteria
  - *Bartonella*
  - *Brucella*
  - *Rickettsia*
- Betaproteobacteria
  - *Bordatella*
  - *Burkholderia*
  - *Neisseria*
- Epsilonproteobacteria
  - *Campylobacter*
  - *Helicobacter*

Important Genera

Proteobacteria

- Gammaproteobacteria
  - Aeromonadaceae
    - *Aeromonas*
  - Enterobacteriales
    - *Escherichia*
    - *Salmonella*
    - *Shigella*
    - *Yersinia*
  - Legionellales
    - *Coxiella*
    - *Legionella*

- Gammaproteobacteria
  - Pasturellales
    - *Pasturella*
    - *Haemophilus*
  - Pseudomonadales
    - *Pseudomonas*
  - Thiotrichales
    - *Francisella*
  - Vibrionaceae
    - *Vibrio*

Important Genera

- Spirochaetes
  - *Leptospira*
  - *Borrelia*
  - *Treponema*

Bacillus anthracis: Anthrax

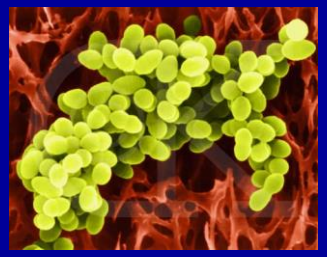
- Large, gram positive non-motile rod
- Vegetative form and spores
- Nearly worldwide distribution
- Over 1,200 strains
- 5th Plaque of Bible
- Spores 1.5-3 microns

Epidemiology

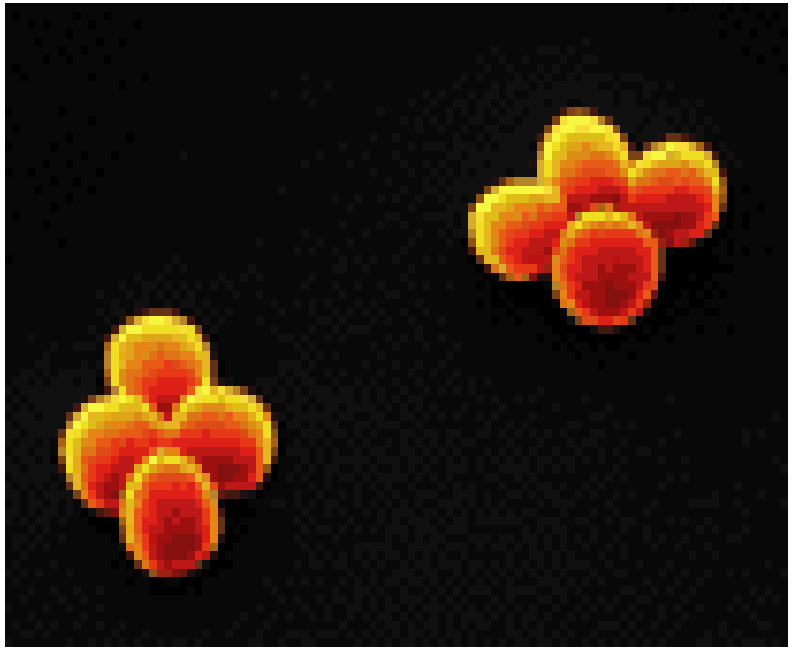
- Three forms of natural disease
  - Inhalational
    - Rare (<5%)
    - Most likely encountered in bioterrorism event
    - 86-100% Mortality (despite treatment)
  - Cutaneous
    - Most common (95%)
    - Direct contact of spores on skin
    - <5% (treated) – 20% (untreated) mortality
  - Gastrointestinal
    - Rare (<5%), never reported in U.S.
    - Ingestion
    - approaches 100% mortality

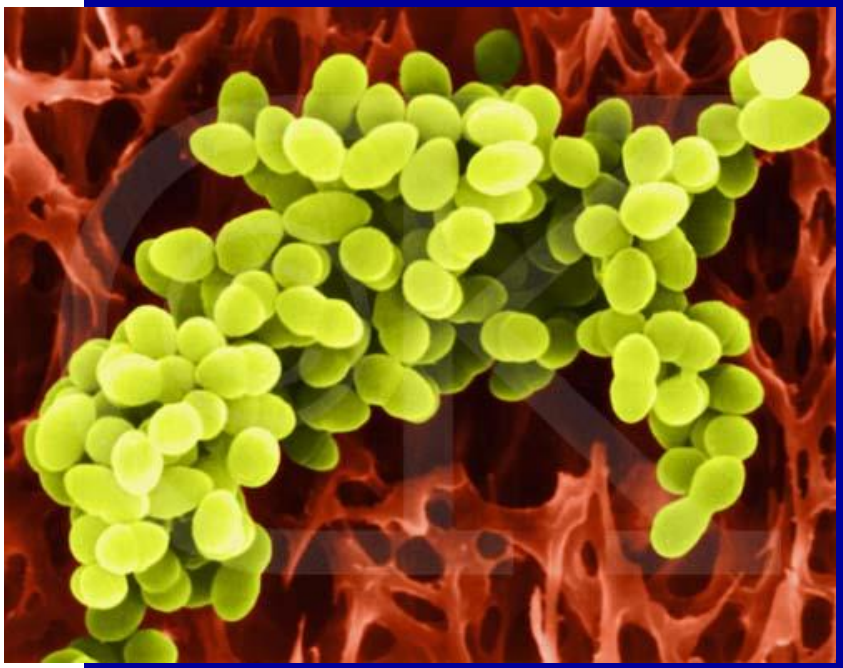
Staphylococcus aureus

- Gram positive, aerobic cocci
- Staph are found in air, dust, sewage, water milk, food, equipment, animals and humans: skin, hair, nose, throat, open sores, boils, saliva
- Transmitted to foods via handling, coughing, sneezing, wiping

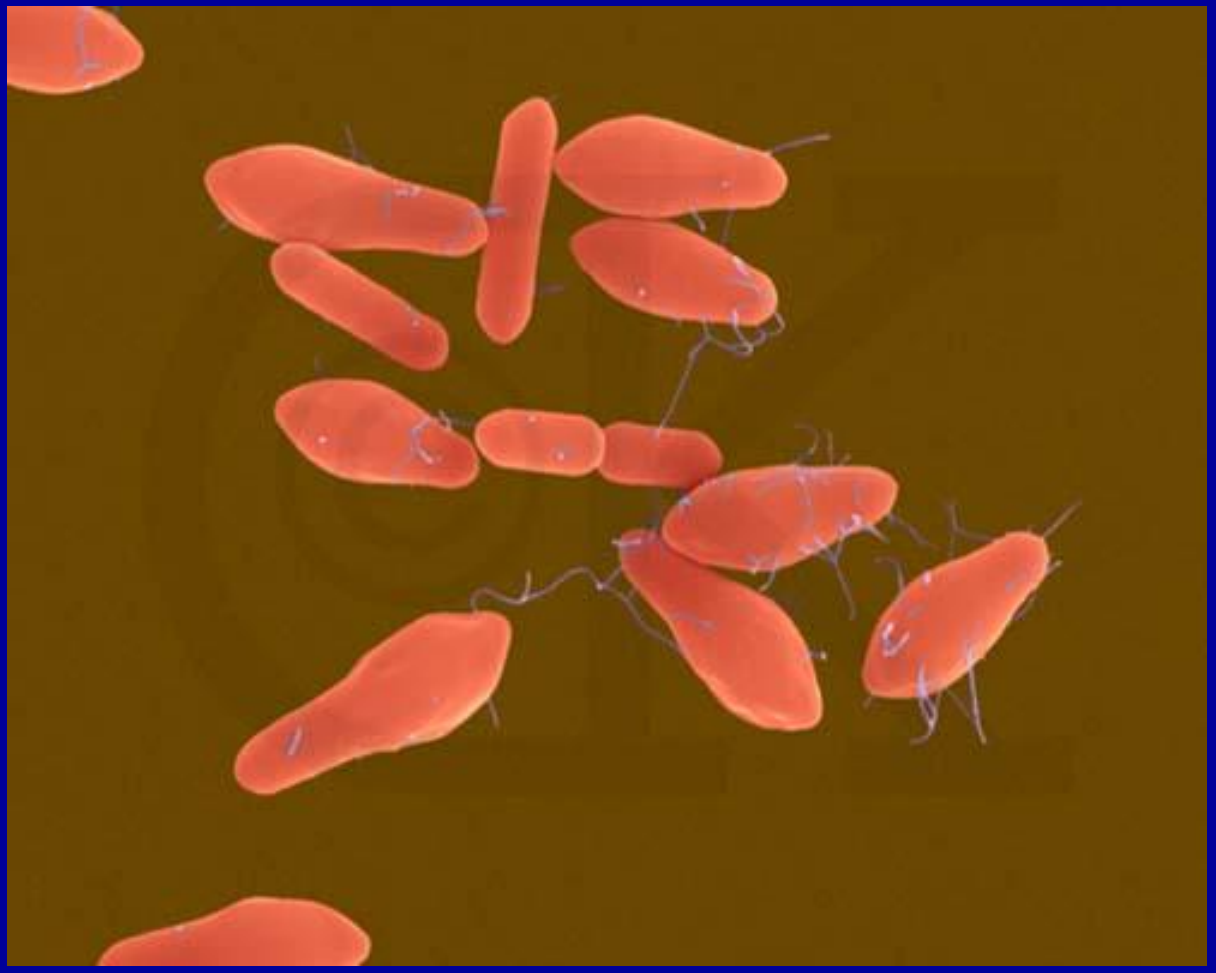


Staphylococcus aureus



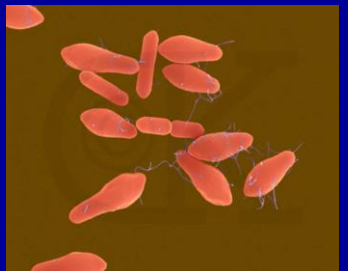


Clostridium perfringens



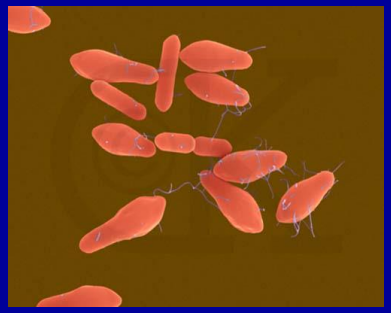
Clostridium perfringens

- Gram positive, spore forming, anaerobic rod
- Found in soil, intestinal tracts of man and animals
- Foodborne infection;8-22 hours; toxin is formed in the gut
- Symptoms: Diarrhea, severe dehydration, cramps



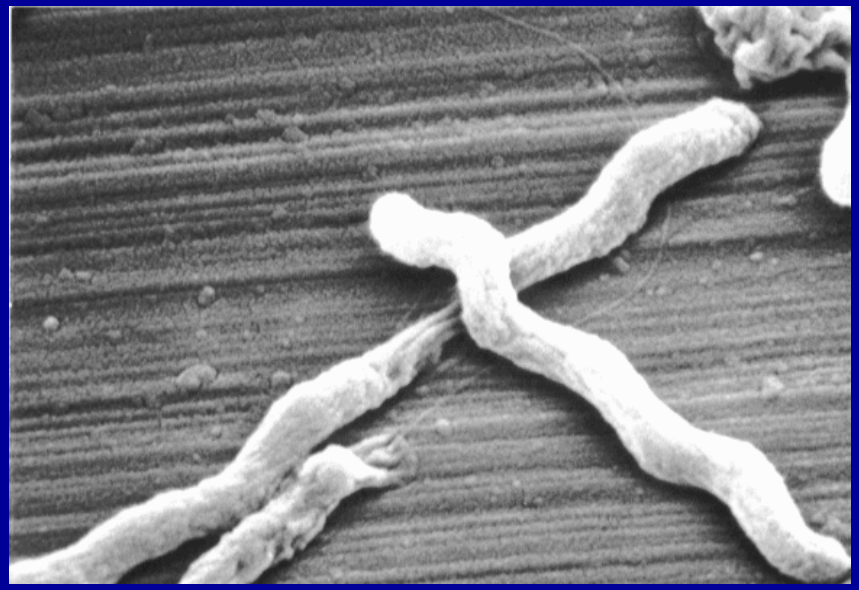
Clostridium perfringens

- Large number of cells (10^8) needed to cause disease
- Associated foods: temperature abused foods, roast beef, stews, meat gravy, poultry



Campylobacters

- Gram-negative
- Curved rod
- about 1.5-3 microns
- motile via polar flagella
- Microaerophilic
- Prefer high CO_2



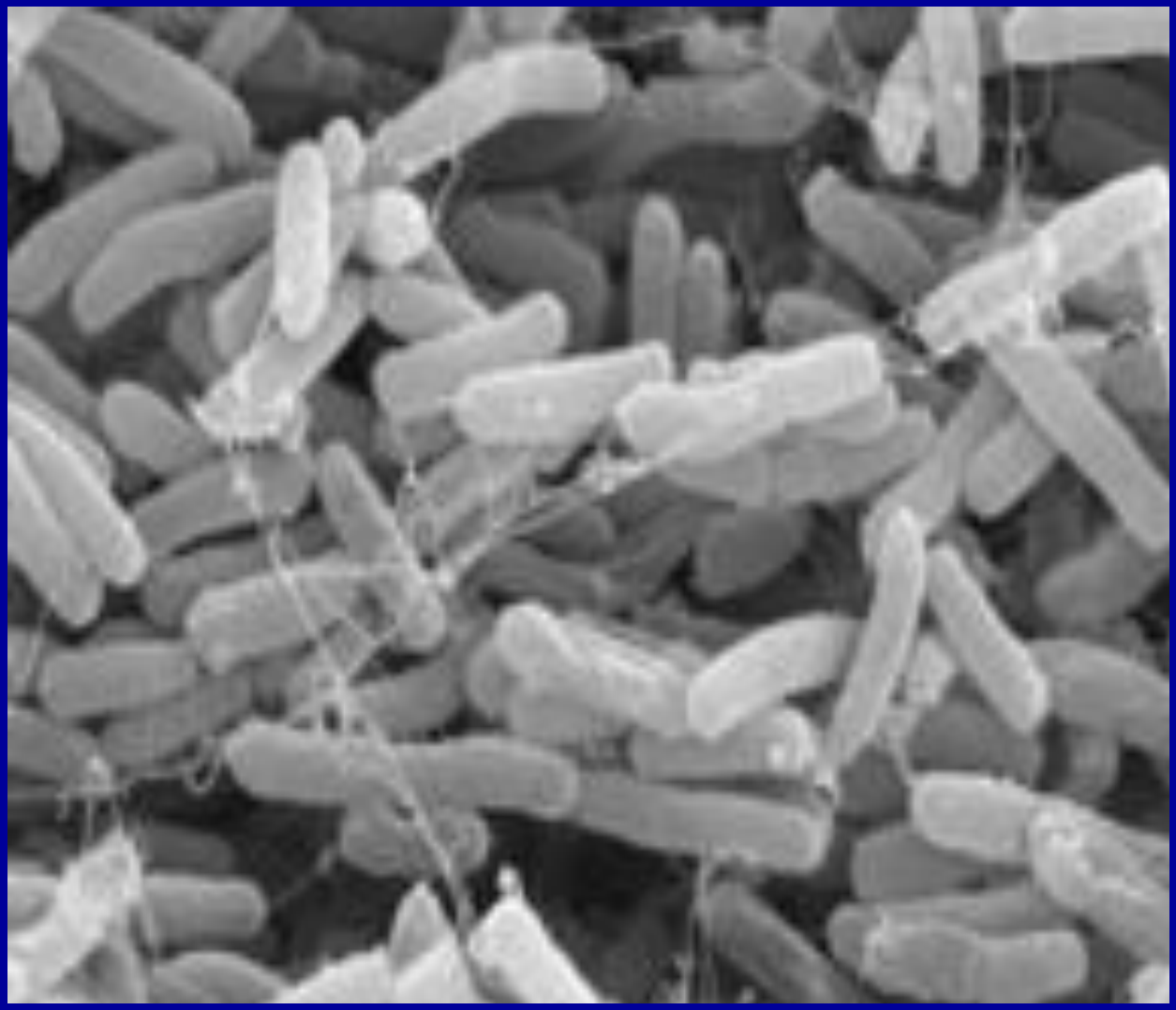
Complications and Sequelae of Campylobacteriosis; Guillain-Barré Syndrome

- Develop a rare disease of the nervous system beginning several weeks after the diarrheal illness.
- Called Guillain-Barré syndrome
- Person's immune system is "triggered" to attack the body's own nerves
- can lead to paralysis lasting several weeks; usually requires intensive care
- About 1 per 1000 reported campylobacteriosis cases leads to Guillain-Barré syndrome.
- Perhaps 40% of Guillain-Barré syndrome cases in this country may be triggered by campylobacteriosis.

Escherichia coli cells: ~0.5 x 1.0 micrometers

Typical rod-shaped bacteria:

fecal indicator and pathogenic strains



E. coli Genetics and Serology

Genetics:

- Single, circular DNA molecule, ~4 x 10^6 base pairs
- Molecular weight of 4 x 10^9
- Total length of about 1.4mm.

Serology:

- *E. coli* can be subdivided by somatic (cell-wall) or O antigens and flagellar or H antigens.
  - >160 recognized O types and 55 recognized H types
  - over 8000 possible OH serotypes.
  - also capsular (K) and fimbrial antigens.

Virulence Properties of *E. coli*

Enterotoxins:

- at least two types: Heat Stable (ST) and Heat Labile (LT)
- Verotoxins or Shiga-like toxins (interchangeable terms):
  - Verotoxin term is based on the reactions of toxins on Vero cells
    - VT1 (SLT I): similar to Siga-toxin (produced by some strains of Shigella dysenteriae)
    - VT2 (SLT II) which is only about 50% realted Shiga toxin.
- Other Toxins:
  - Cytolethal distending toxin (CLDT), VirCytotoxin, Cytotoxic necrotising factors (CNF), a possible Enteropathogenic E. coli EPEC) enterotoxin and a possible E. coli Sudden Infant Death Syndrome (SIDS)-toxin.
- Haemolysins:
  - extracellular haemolysin known as alpha-haemolysin (many strains)
  - cell-associated haemolysin, beta-haemolysin, (some strains)
  - enterohaemolysin: extracellular; Enterohaemorrhagic E. coli (EHEC)

Pathogenic *E. coli*

Enteric Infections:

- Enteroadherent *E. coli* (EAEC)
- Enteroaggregative *E. coli* (EAggEC)
- Enterohaemorrhagic *E. coli* (EHEC)
- Enteroinvasive *E.coli* (EIEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC)

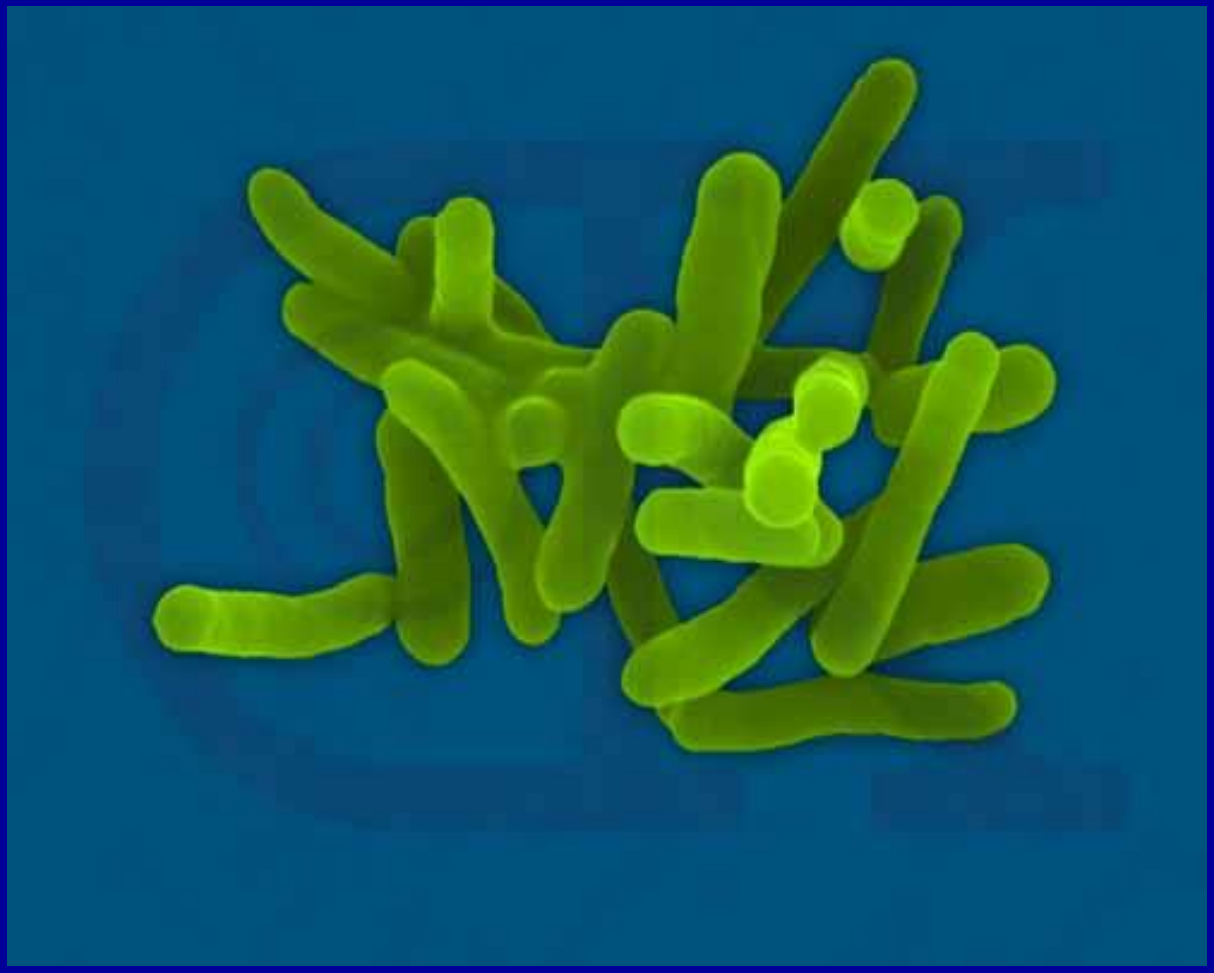
Extraintestinal Infections:

- Uropathogenic *E. coli* (UPEC): urinary tract infections
- Neonatal Menigitis *E. coli* (NMEC).

Virulence Properties of *E. coli*

- Fimbriae: CFAI/CFAII, Type 1 fimbriae, P fimbriae, S fimbriae
  - most important: K88, K99 and CFA fimbriae associated with enterotoxigenic *E. coli* (ETEC). They have differing species specificities.
  - The p-fimbriae: associated with urinary tract pathogens.
  - *E. coli* also produce common fimbriae not associated with virulence.
- Adhesins:
  - Intimin: non-fimbrial adhesin; causes the intimate association with target cells in enteropathogenic and enterohaemorrhagic *E. coli* .
    - Associated with the 'attachment and effacement' phenomenon
    - Causes destruction of the intestinal surface cells.
    - Other outer membrane proteins can act as adhesins.

Shigella spp.



Shigella and Shigellosis

- Fecal-oral transmission
  - person-to-person, fomites, food, water, ect.
- Waterborne and water-washed
- Reservoirs: humans and primates
- Infectious dose: low; as few as 10 cells to infect
- Incubation period: 1 to 7 days; typically, 1-3 days
- Duration of illness:
  - untreated: severe symptoms for about two weeks
  - Antibiotic treatment shortens illness and prevent spread to others

Shigellosis - Epidemiology

- Four species of *Shigella*: *flexneri, sonnei, dysenteriae, boydii*
- Major public health problem in many developing countries
  - causes about 5 to I0% of childhood diarrhoea
  - up to 25% of all diarrhea-related deaths can be associated with Shigella

Developing countries:

- Sh. *flexneri* is endemic (always present) in most communities
- Sh. dysenteriae type 1 often occurs in an epidemic pattern
  - organism can be absent for a number of years, then reappear and infect a large proportion of the population.
- These two species of Shigella generally produce the most severe illness.

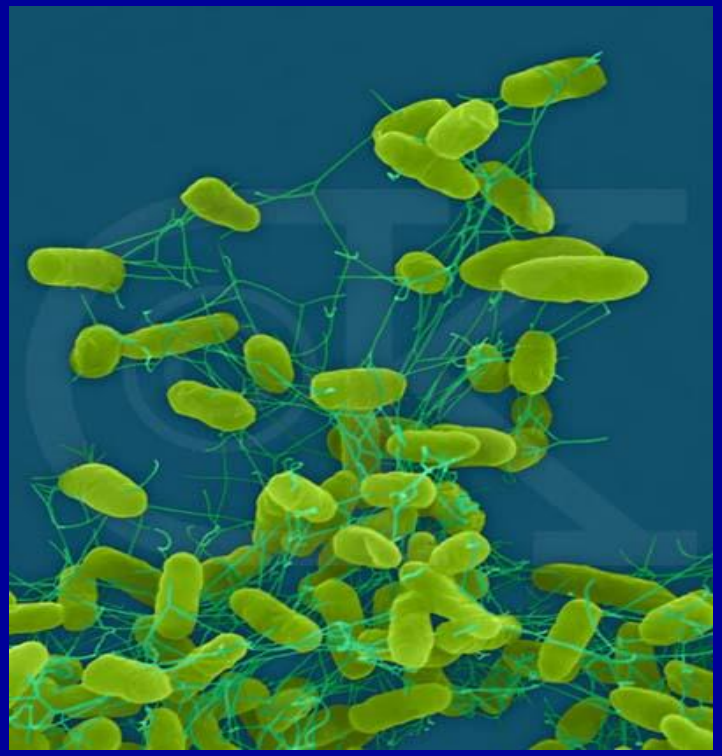
Developed countries:

- *Sh. sonnei* is the most common and is the least virulent
- Sh. boydii causes disease of intermediate severity
  - is least common, except in the Indian sub-continent.

Salmonella and Salmonellosis

- Belong to Enterobacteriaceae family
- Gram-negative bacilli; facultative and flagellated (motile).
- 3 major antigens:
  - "H" or flagellar antigen (phase 1 & 2)
  - "O" or somatic antigen (part of the LPS moiety)
  - "Vi" or capsular antigen (called "K" in other Enterobacteriaceae).
- **Posess LPS endotoxin characteristic of Gram-negative bacteria**
  - composed of an "O" polysaccharide ("O" antigen)
  - "R" core
  - endotoxic inner "Lipid A".
  - Endotoxins evoke fever and can activate complement, kinin and clotting factors.

Salmonella spp.

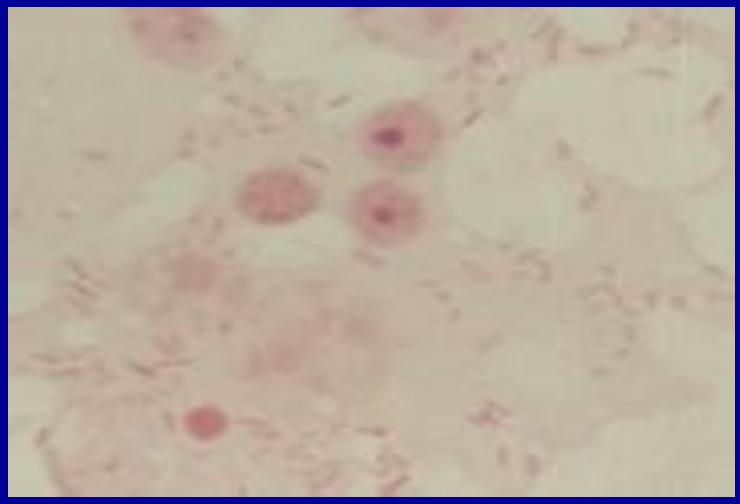


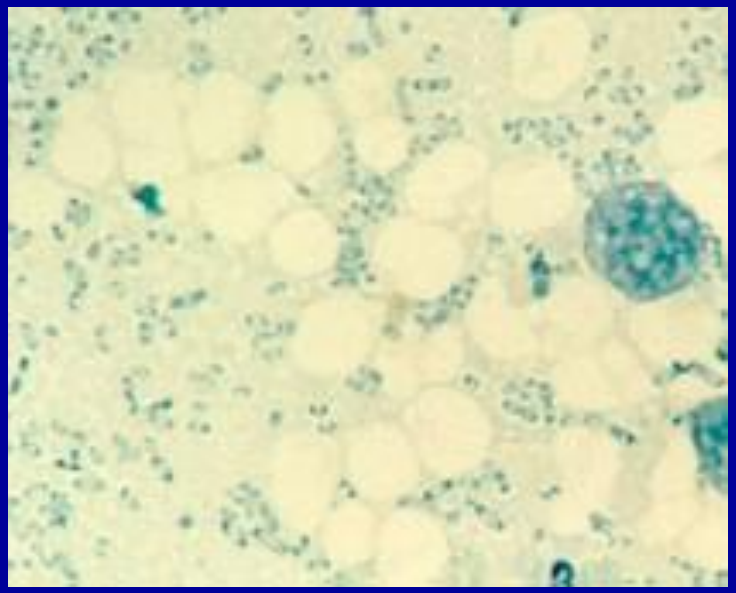
Typhoid fever: (*S. typhi* and *S. paratyphi):* Systemic Infection

- Fecal-oral transmission
- Systemic infection:
  - Macrophages, reticuloendothelial system (esp. liver, spleen and bone marrow), gallbladder and intestines as major sites of damage
- 1.5-2 week incubation period
- Symptoms: fever, headache, malaise, anorexia, then bloody diarrhea
- Mortality rate 10%, if untreated
- **Carrier state possible**
  - **"Typhoid Mary": infamous food handler; infected hundreds**
- Fecally shed at billions/gram by ill persons and carriers

Yersinia pestis

- Gram Stain:
  - Small, gram-negative bipolar-stained coccobacilli
- Wayson Stain:
  - Pink-blue cells with a closed safety pin look





Yersinia pestis: Plague

- U.S. averages 13 cases/yr (10 in 1998)
- 30% of cases are in Native Americans in the Southwest. 15% case fatality rate
- Most cases occur in summer

Plague Epidemiology

- Three Clinical Types:
  - bubonic (infected lymph nodes)
  - septicemic (blood-borne organisms)
  - pneumonic (transmissible by aerosol; deadliest)

Legionella spp.

- Gram-negative
- Aerobic
- Non-sporing
- Encapsulated
- ~46 species, 68 serogroups
- Ubiquitous aquatic organism
- Thrives in warm environments (32C-45C)

Legionella: Legionellosis and Pontiac Fever

Reservoirs and amplifiers:

- Hot water systems
- circulating water ventilation systems (cooling towers)
- Plumbing (e.g., shower heads).
- Hot tubs, whirlpools, etc.
- Produce fresheners

Cleveland Auto plant outbreak, March, 2001:

- Plant cooling tower is considered a possible source of the outbreak.
- But, more than 100 other internal water sources -- favorite breeding grounds for the Legionella bacteria -- were also under investigation….

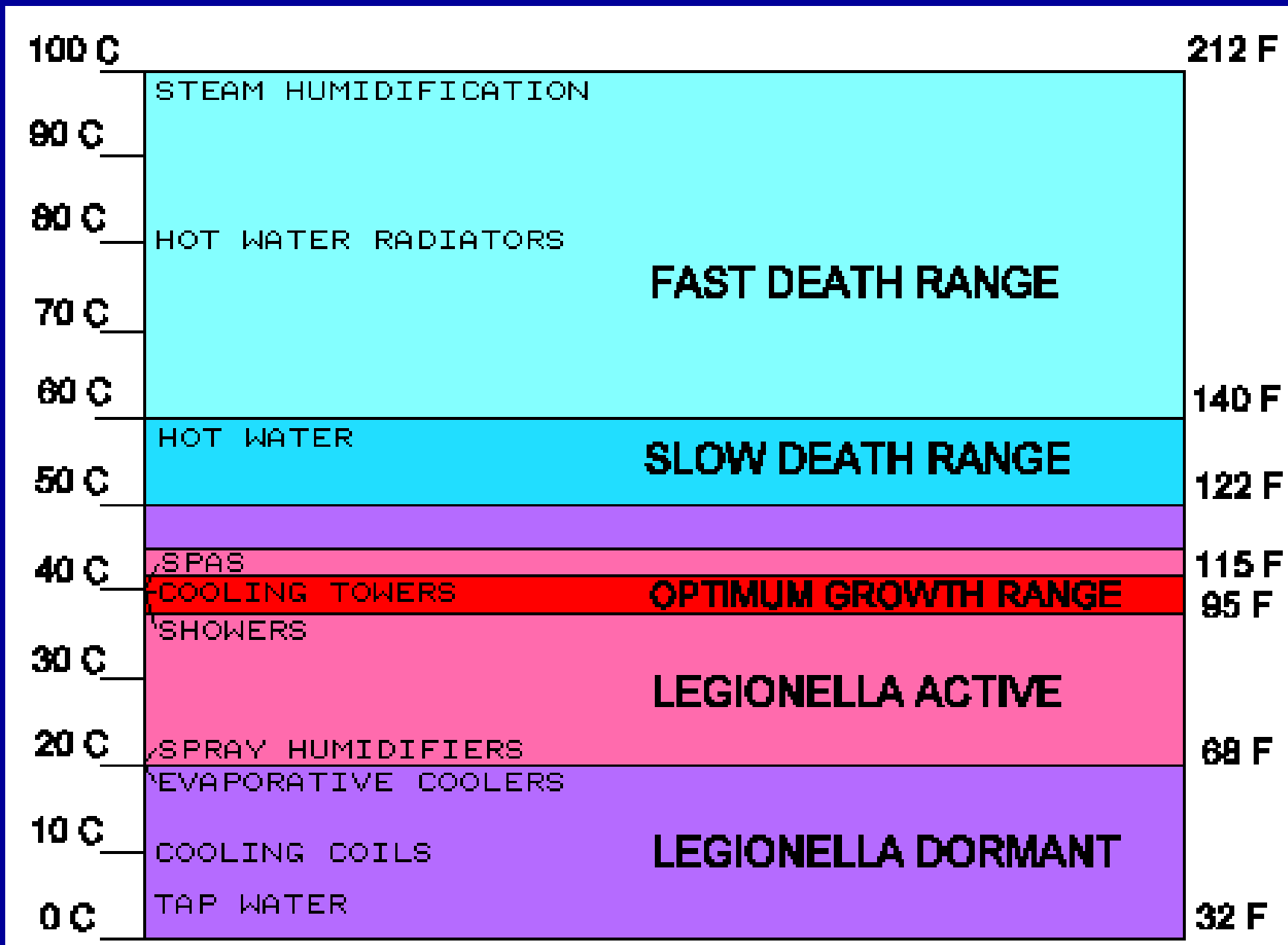
Legionnaire's Disease and Pontiac Fever

Legionnaire's disease:

- Bacterial pneumonia caused by *Legionella pneumophila*.
- A type of pneumonia that affects the lungs and may also affect the stomach and intestines, kidneys, and central nervous system.
- Incubation period: 2-10 days after exposure
- Frequently requires hospitalization
- Aerosol exposure from contaminated cooling towers, evaporative condensers, whirlpools, shower heads, faucets, & hot water tanks.

Pontiac fever: also caused by Legionella.

- A "flu-like" illness with fever, chills, headache, myalgia (pain in the muscles), cough, nausea, and breathlessness.
- Pneumonia does not occur.
- Usually lasts 2-5 days.
- Same sources as for Legionnaires' disease


100 C
212 F
STEAM HUMIDIFICATION
90 C
80 C
HOT WATER RADIATORS
FAST DEATH RANGE
70 C
60 C
140 F
HOT WATER
SLOW DEATH RANGE
50 C
122 F
SPAS
40 C
115 F
COOLING TOWERS
OPTIMUM GROWTH RANGE
95 F
SHOWERS
30 C
LEGIONELLA ACTIVE
20 C
SPRAY HUMIDIFIERS
68 F
EVAPORATIVE COOLERS
10 C
COOLING COILS
LEGIONELLA DORMANT
TAP WATER
0 C
32 F