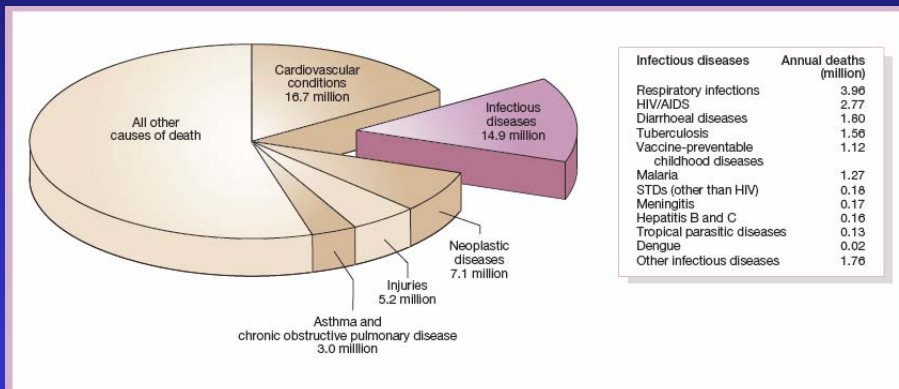


Microbiology/Infectious Diseases Course



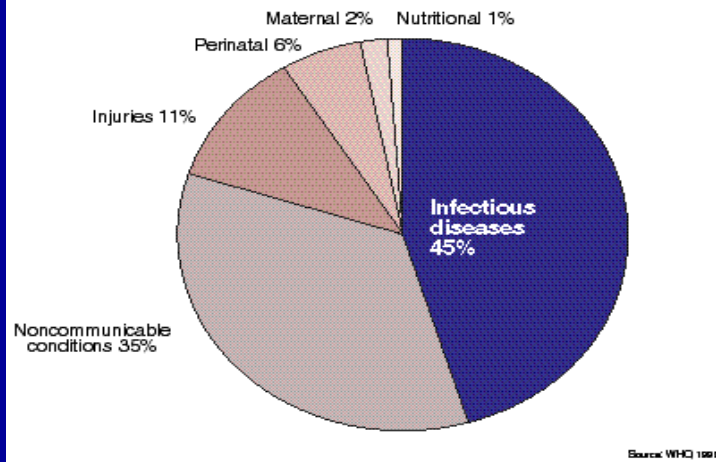
Welcome!

Leading Causes of Death Worldwide



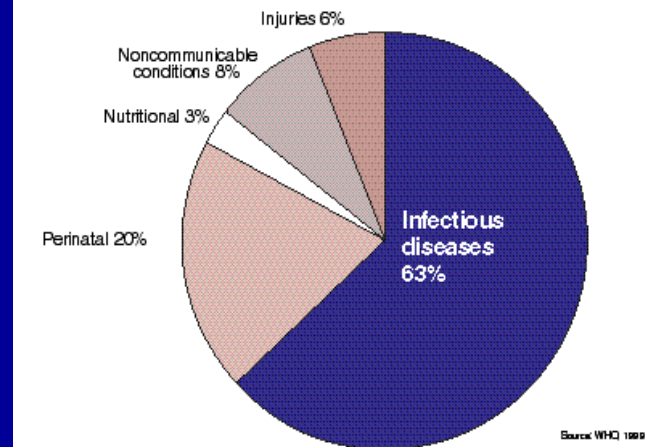
Main causes of death in low-income countries

In South-East Asia and Africa
Estimates for 1998



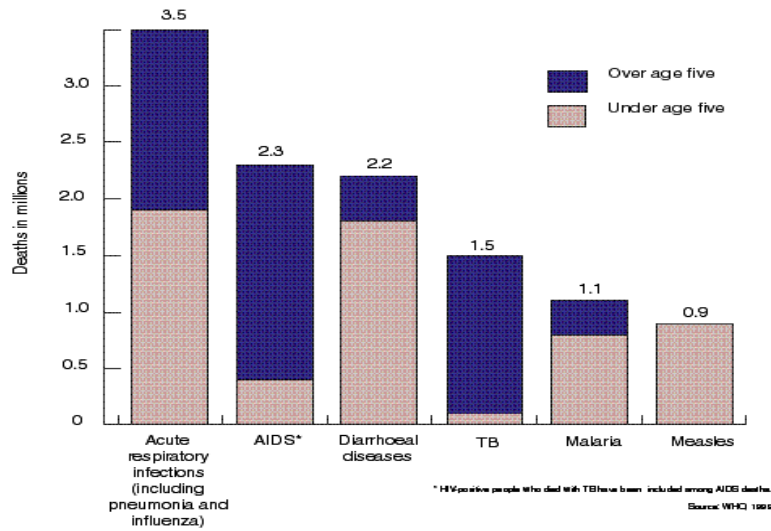
Main causes of death among children

Ages 0 to 4 years
Estimates for 1998, worldwide

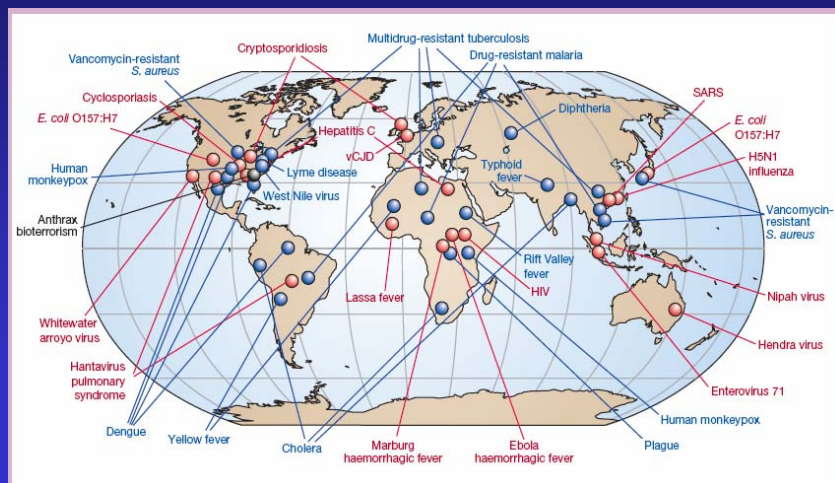


Leading infectious killers

Millions of deaths, worldwide, all ages, 1998



Global Examples of Emerging or Re-emerging Infectious Diseases



Subjects to be Covered - Lectures 1&2

- General introduction to the Microbiology/Infectious Diseases course
- Bacterial classification systems
 - Phenotypic and genotypic systems
- Description of medically important bacterial pathogens
- Bacterial structure
 - Bacterial components and secreted products
- Distinction between Gram positive and Gram negative bacteria

Purpose of Classification

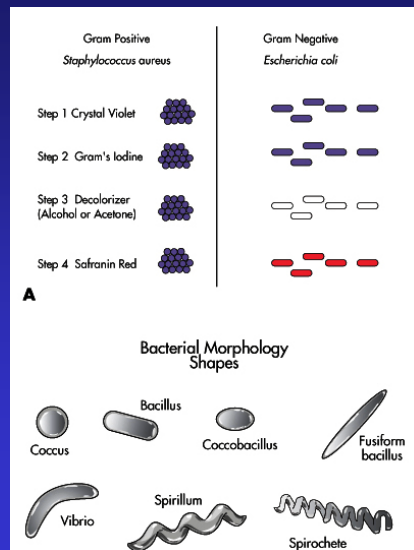
“The primary purpose of nomenclature of microorganisms is to permit us to know as exactly as possible what another clinician, microbiologist, epidemiologist or author is referring to when describing an organism responsible for infection of an individual or outbreak.”

S. Finegold
1993

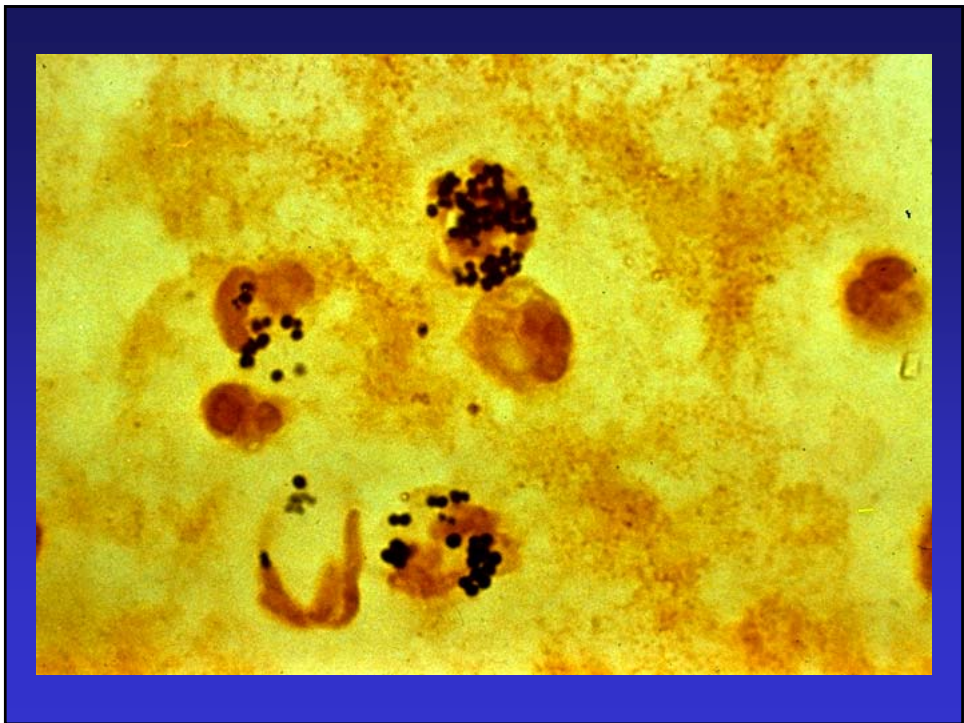
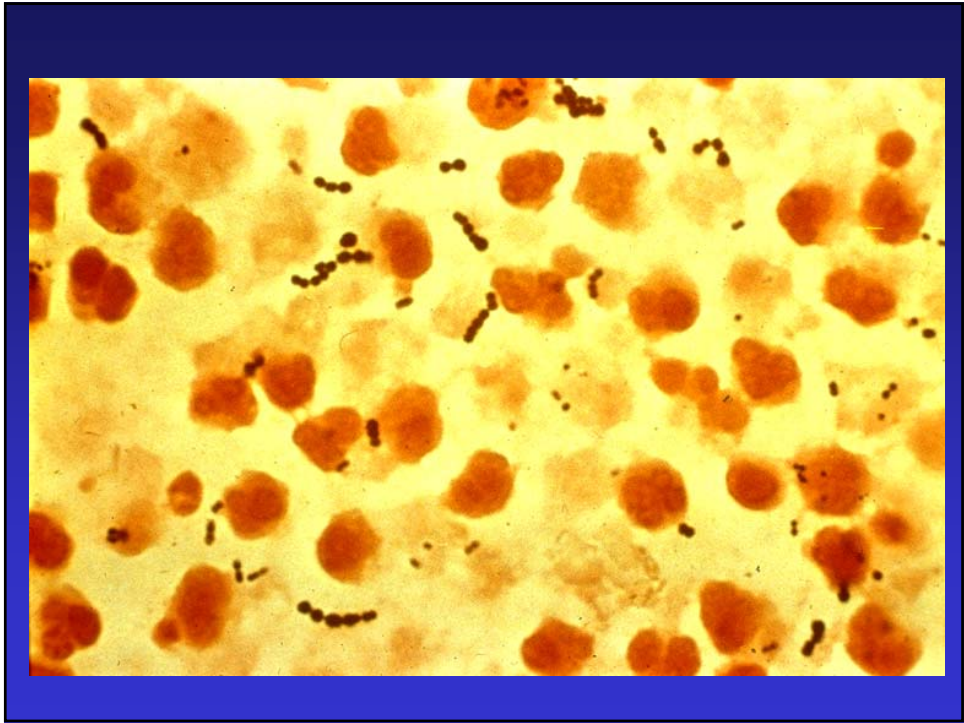
Classification of Bacteria

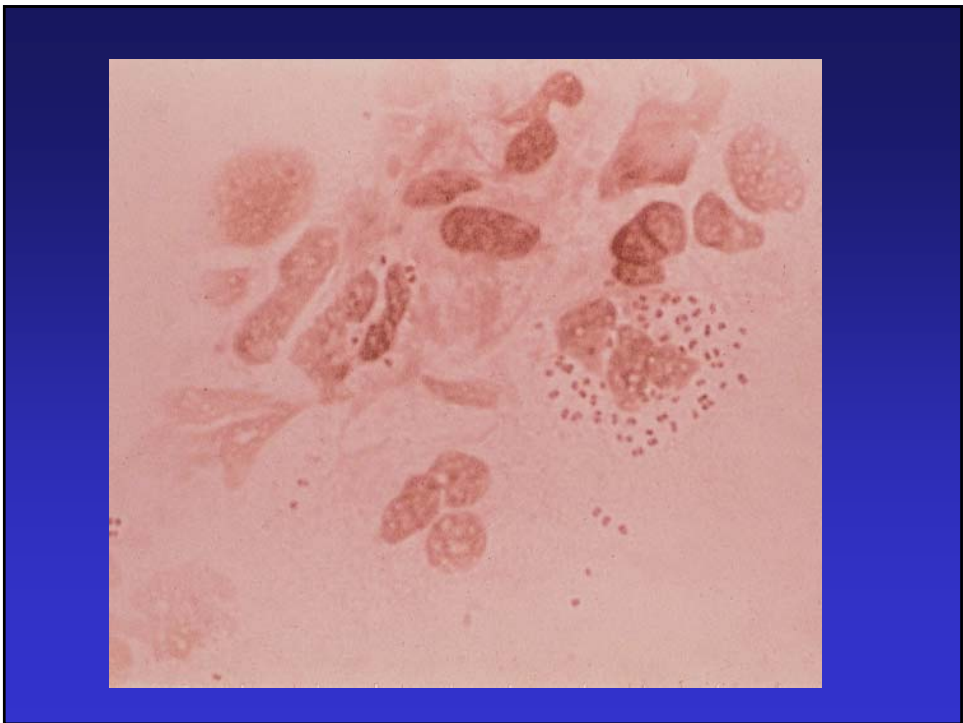
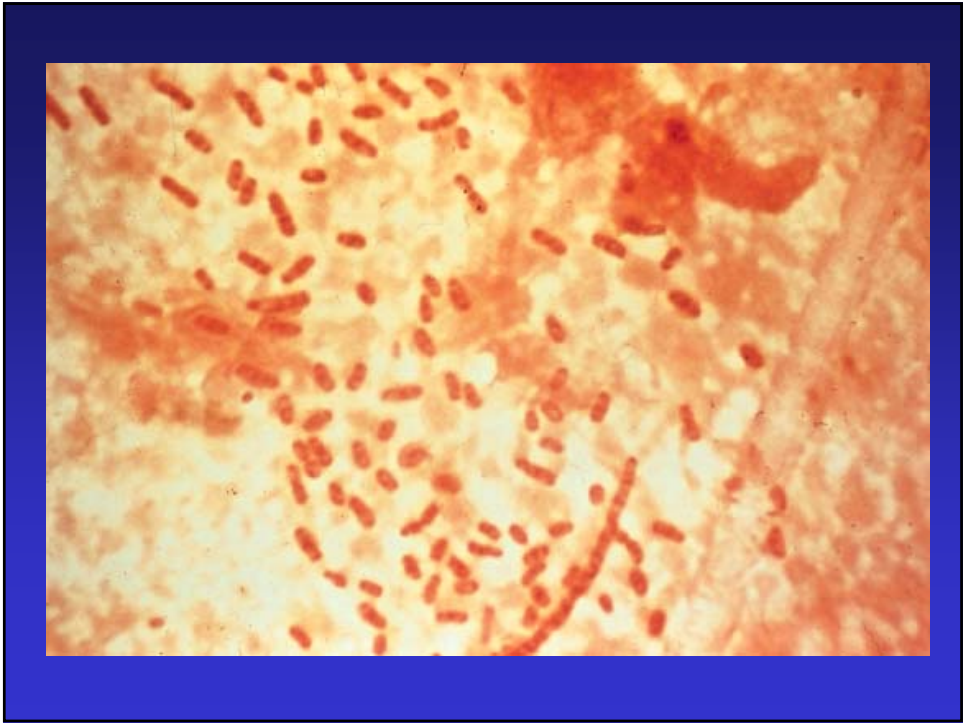
- Classification systems: types and purpose
- Phenotypic classification: Gram stain, morphology, growth requirements and biochemical profiles
- Environmental reservoirs / Modes of transmission
- Genotypic classification: rRNA analysis, strain classification

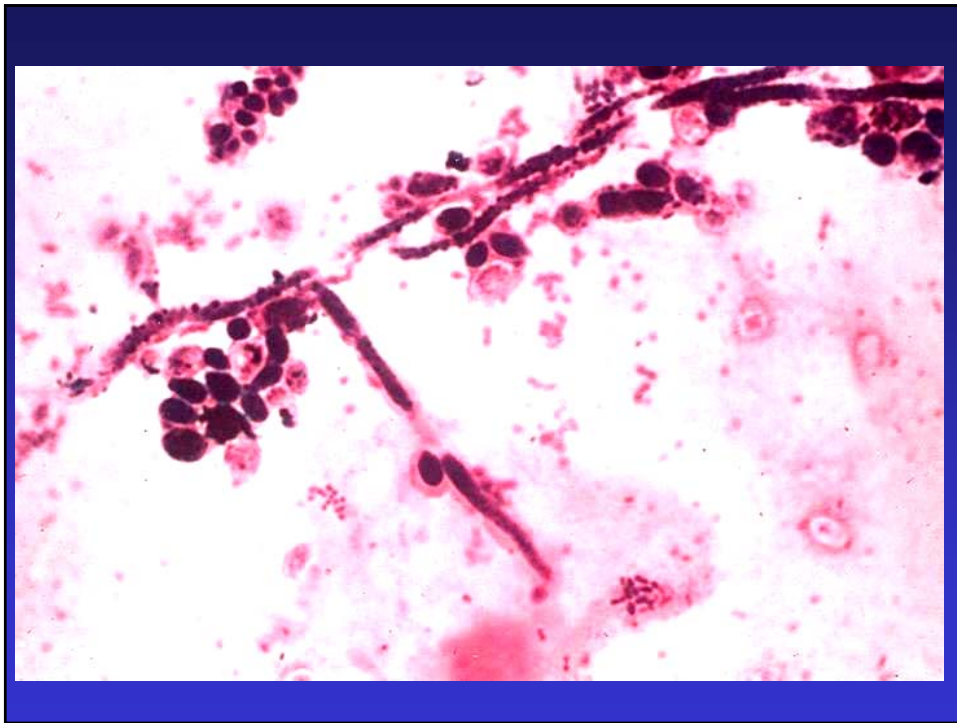
Gram Stain and Bacterial Morphology



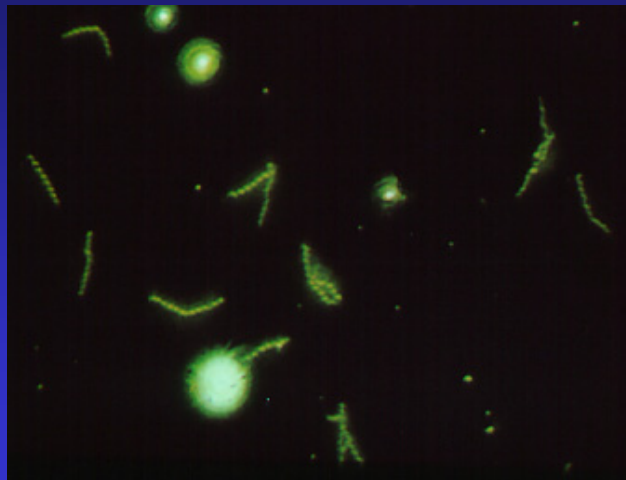
- Stain discovered by H. C. Gram in 1884 remains the most universally used technique to visualize bacteria
- Differences in the peptidoglycan of Gram positive (purple) and negative (red) bacteria responsible for differences in their respective staining properties
- Iodine causes crystal formation in Gm+ entrapping stain. In Gm- alcohol dissolves membrane releasing stain



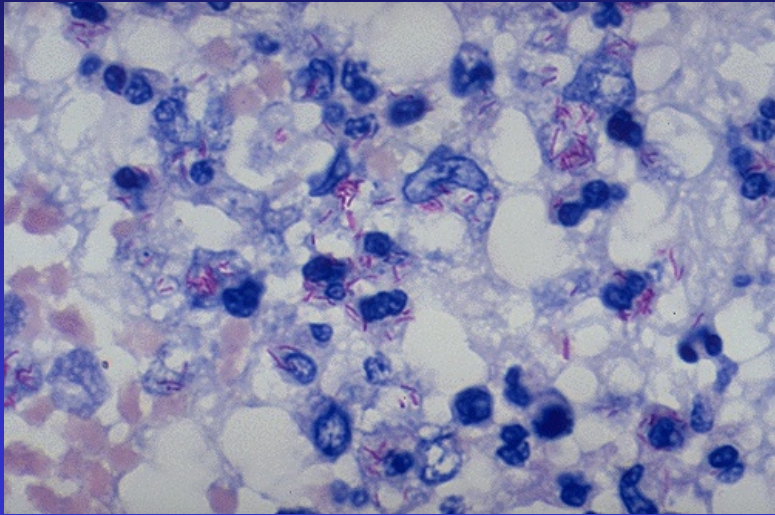




Dark Field Demonstration of Treponemes

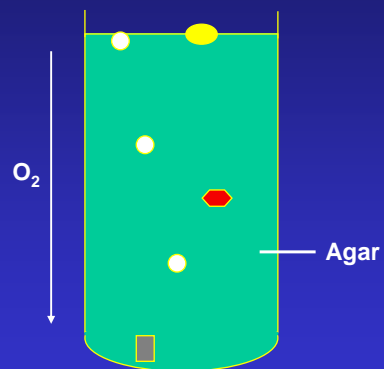


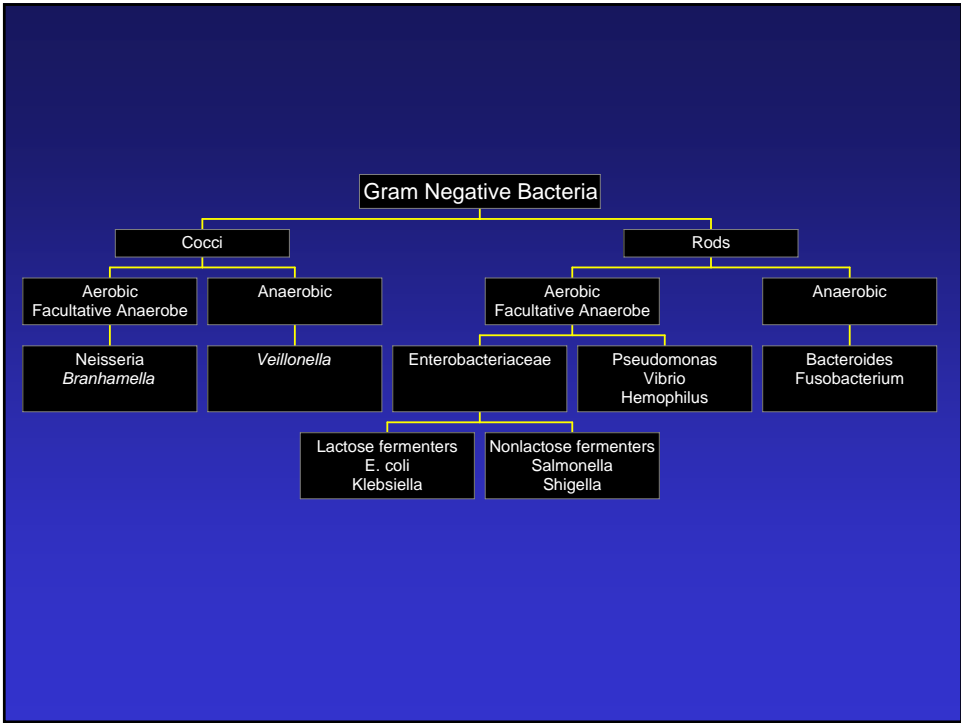
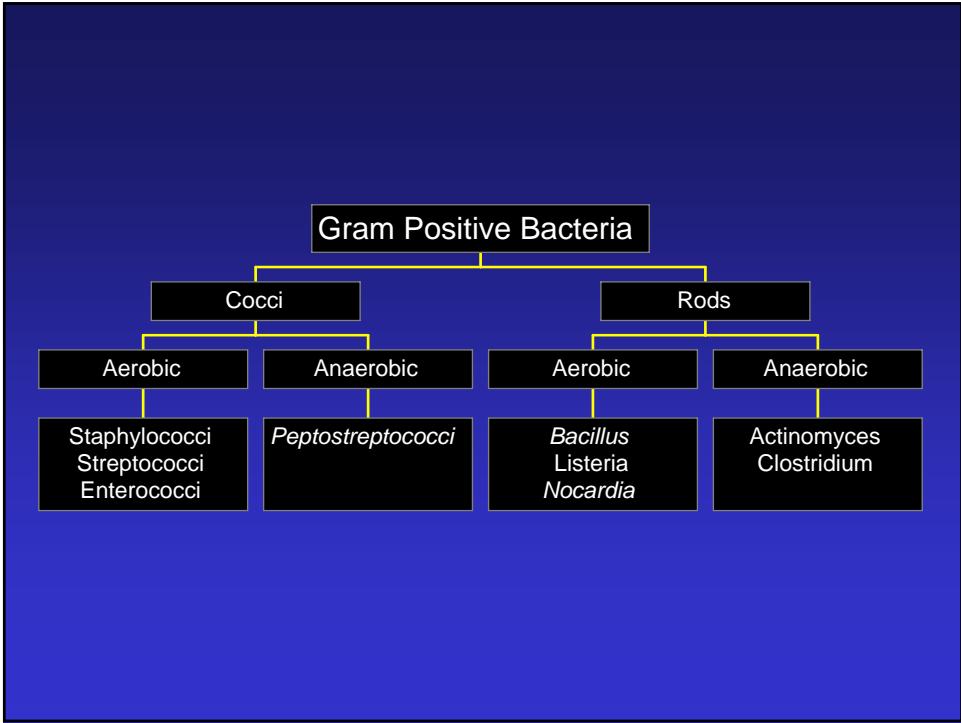
Acid Fast Stain of Mycobacteria

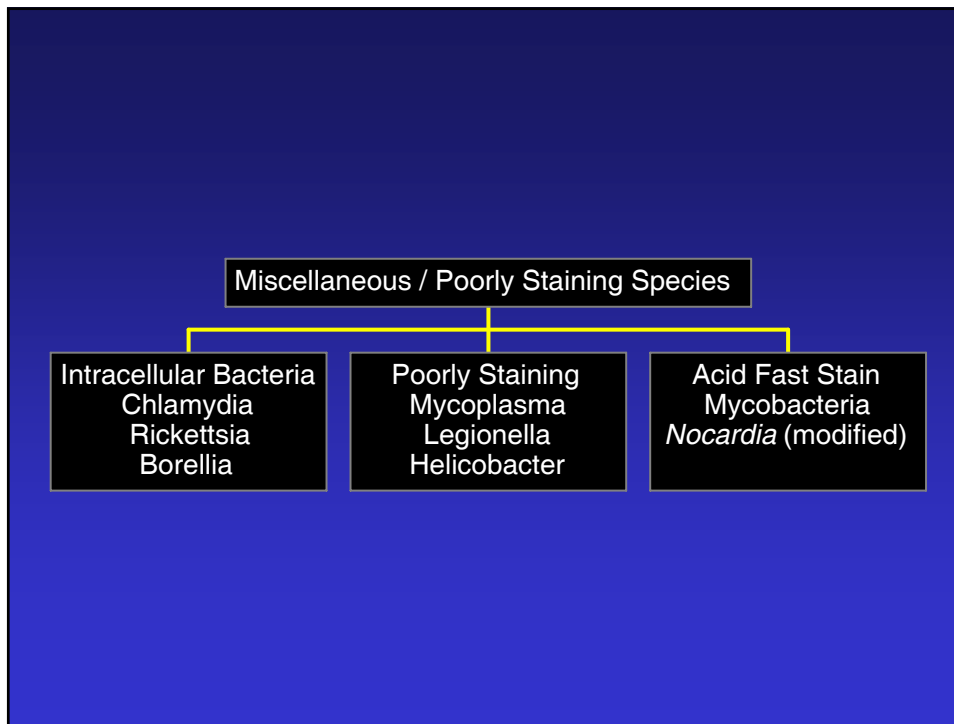


O₂ Growth Requirements of Bacteria

- Facultatively anaerobic:
Escherichia
- Aerobic:
Pseudomonas
- Anaerobic:
Clostridia
- ◊ Microaerophilic:
Campylobacter



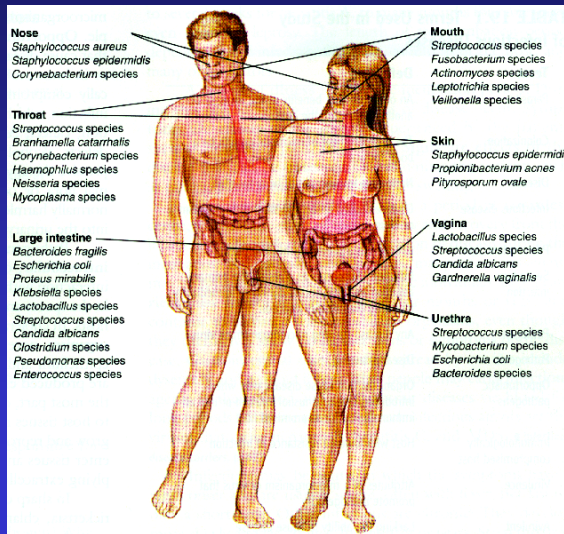




Environmental Bacterial Reservoirs

- Endogenous sites: normal flora
 - Skin - coagulase negative staphylococci
 - Colon - *Escherichia coli*, *Bacteroides spp.*
 - Oropharynx - viridans streptococci
- Exogenous sources of bacterial spread
 - Water - legionella, cholera
 - Air, fomites - *M. tuberculosis*, *B. anthracis*
 - Food - *Salmonella spp.*, *E. coli*
 - Ticks - *Borrelia*, *rickettsia*

Normal Flora

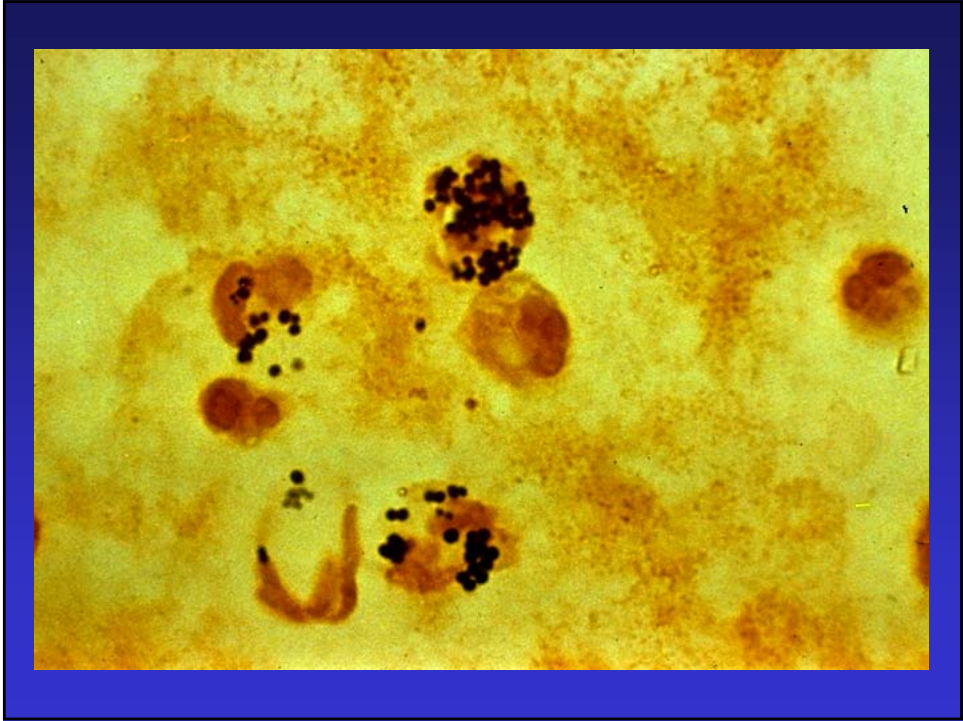


Normally Sterile Body Sites

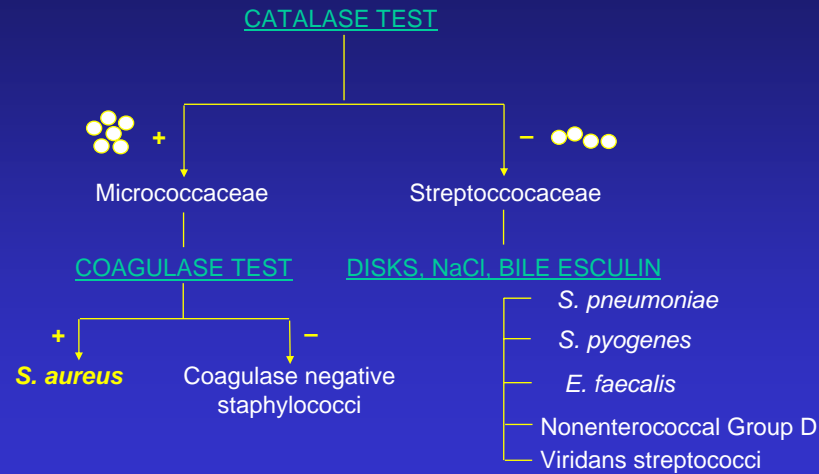
Bloodstream
Bladder
Central nervous system
Lower respiratory tract
Sinuses

The Infected Intravenous Catheter Site

A 73 year old female successfully undergoes coronary artery bypass graft surgery. Two days after the procedure she develops fever, chills and pain at her intravenous line site. Examination reveals marked redness and swelling at the site. Pus is expressible from the catheter line site.



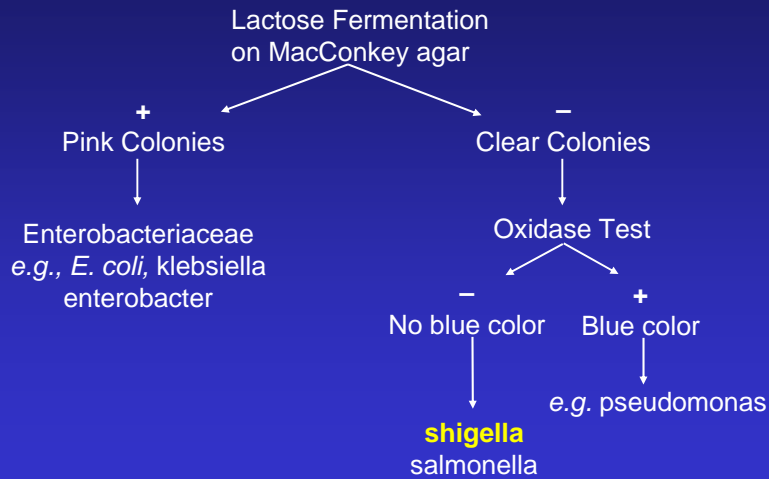
Identification of Aerobic Gram Positive Cocci



A Peace Corps Worker with Diarrhea

A 22 year old Peace Corps volunteer returns from his stint working in a remote village in Senegal. The sanitation conditions in the village were poor. On the flight home he develops watery diarrhea that changes over the next two to three days and becomes bloody. He is sent to your office for evaluation where you find that he is febrile to 103°, dehydrated and has diffuse abdominal tenderness

Identification of Gram Negative Rods



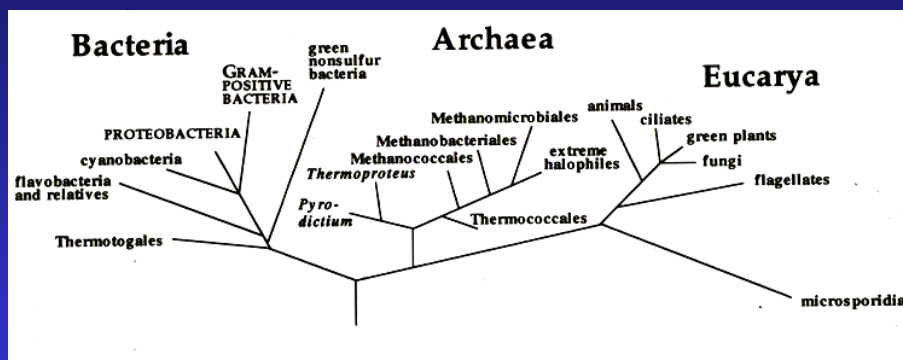
Classification Methods

- Genotypic systems:
 - DNA hybridization - used to designate species
 - Guanine +Cytosine ratio
 - Ribosomal RNA (rRNA) sequence analysis

Ribosomal RNA

- Present in all living cells
- Function of ribosomes is highly conserved
- Mutations are accumulated at a slow, consistent rate
- There are both highly conserved and highly variable regions that are useful for the design of specific PCR primers for phylogenetic analysis

Universal Phylogenetic Tree



Based on model of C.R. Woese

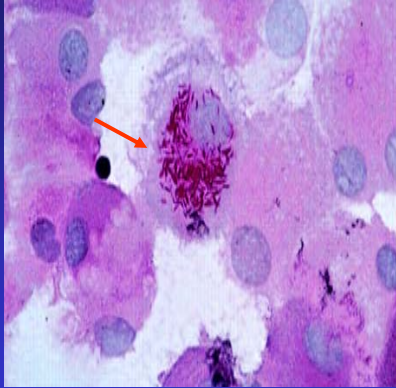
Applications of rRNA Analysis

- < 1% of all microorganisms have been cultured.
- PCR techniques combined with the use of 16S rRNA molecular phylogeny has resulted in the characterization of an increasing number of noncultivable pathogens
- This technique has applications in rapid diagnosis, selection of therapy, as well as phylogenetic classification
- Noncultivable pathogens for selected diseases identified with this technique e.g., Whipples and Cat scratch disease

Use of Ribosomal RNA to Identify Unculturable Bacteria

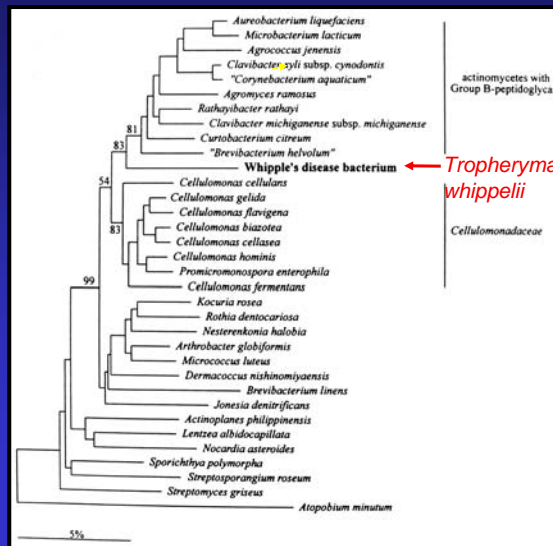
- Harvest DNA from infected tissue
- Amplify 16S rRNA from DNA by PCR using broad range primers
- Determine sequence - from the variable region of amplified sequence determine new primers that are pathogen specific
- Demonstrate that this sequence is clearly associated with the infection
- Determine the evolutionary relationship of the sequence with other known 16S ribosomal sequences
 - Develop diagnostic and therapeutic strategies as a result

The Example of Whipple's Disease



Whipple's disease, a multi-system disease 1st described in 1907, recognized as an infectious disease by PAS staining of tissue. The symptoms include weight loss, diarrhea, abdominal pain, fever and arthropathy. It was among the first noncultivable pathogens characterized by 16S rRNA analysis

Phylogenetic tree for Whipple's Disease Pathogen

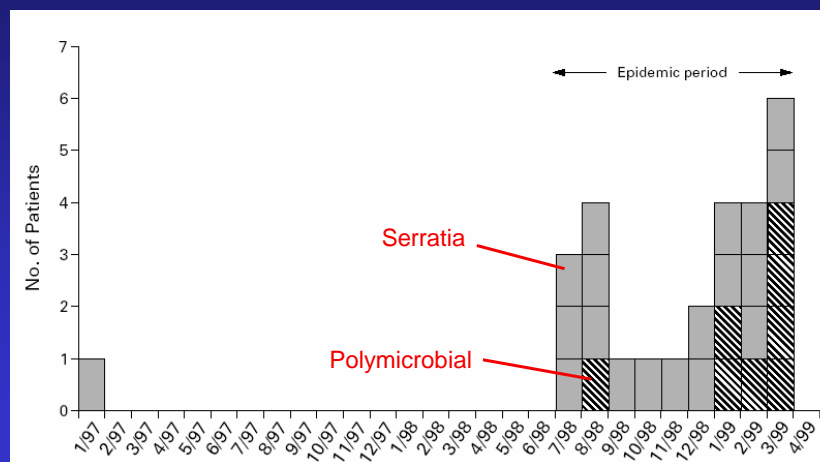


Classification Methods

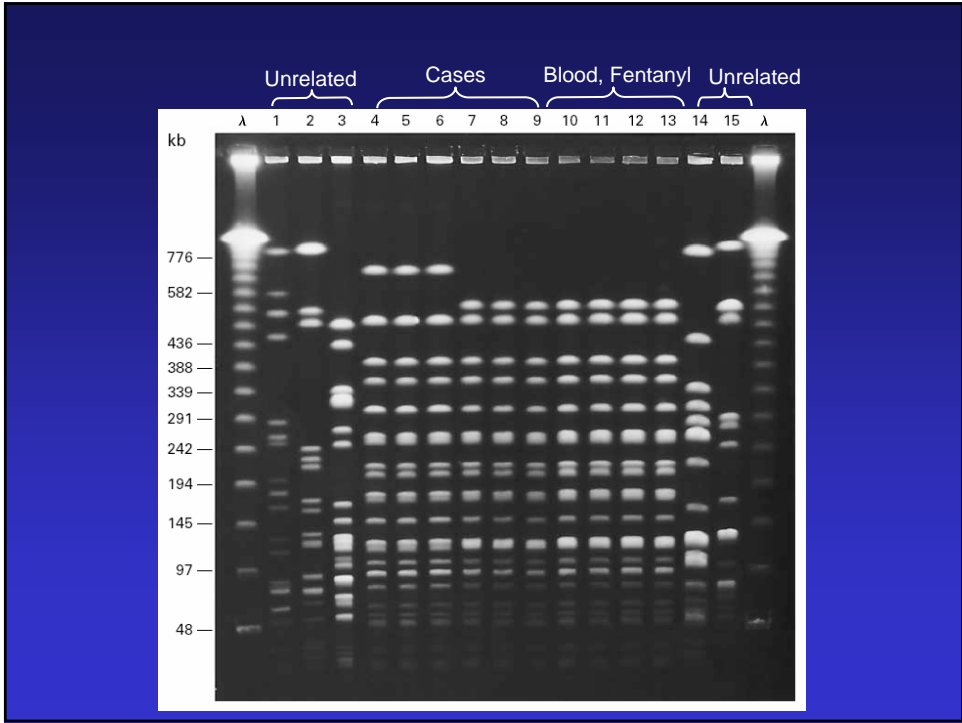
- Molecular subtyping:
 - Pulsed field gel electrophoresis
 - Restriction fragment length polymorphism

SERRATIA MARCESCENS BACTEREMIA TRACED TO AN INFUSED NARCOTIC

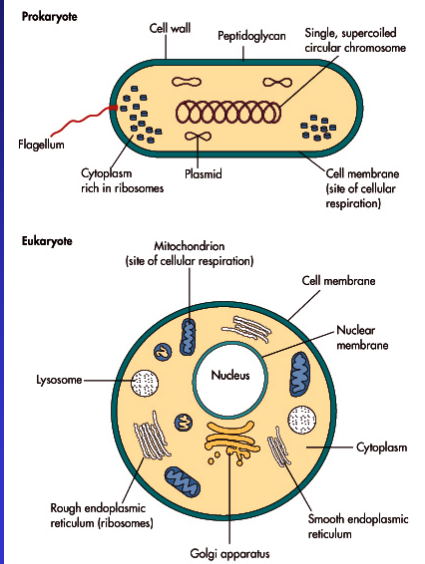
BELINDA E. OSTROWSKY, M.D., M.P.H., CYNTHIA WHITENER, M.D., HELEN K. BREDBERG, M.D.,
LORETTA A. CARSON, M.S., STACEY HOLT, B.S., LORI HUTWAGNER, M.S., MATTHEW J. ARDUINO, DR.P.H.,
AND WILLIAM R. JARVIS, M.D.



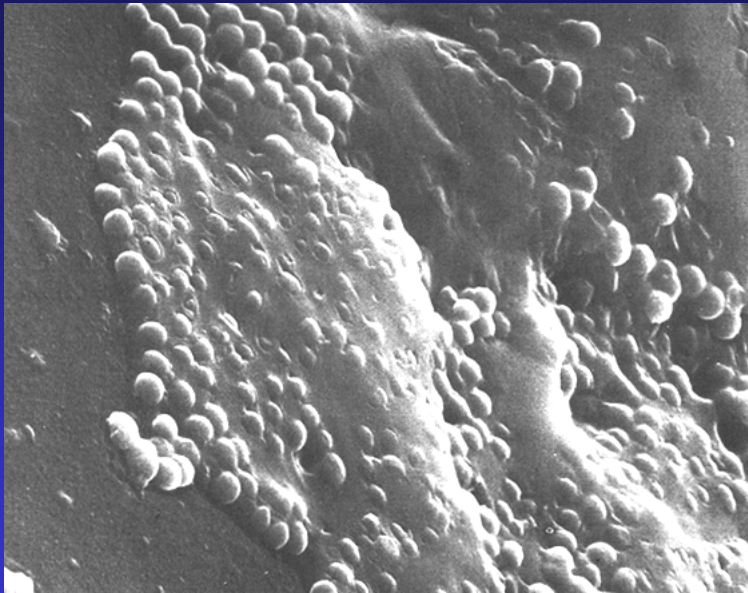
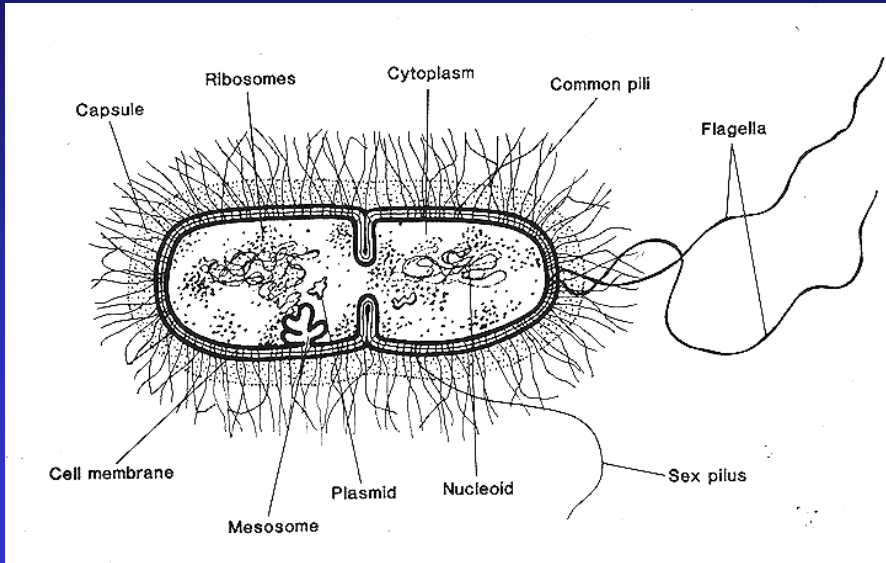
NEJM 346:1529, 2002

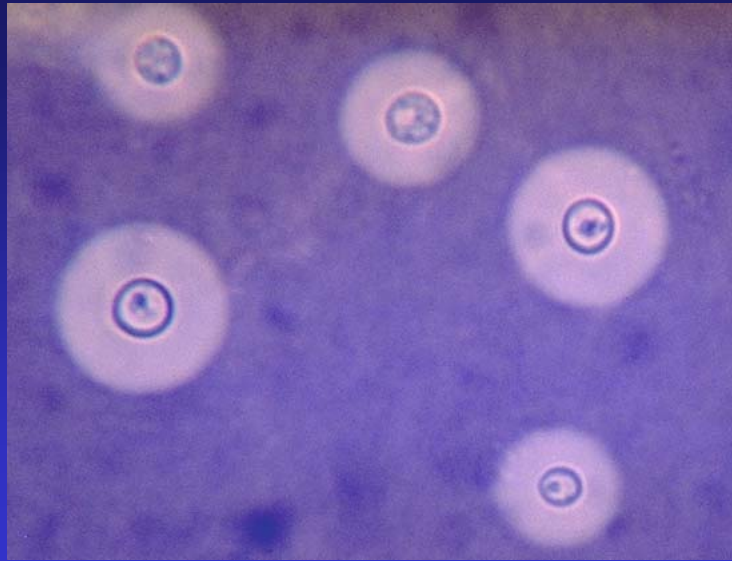


Prokaryotes vs. Eukaryotes

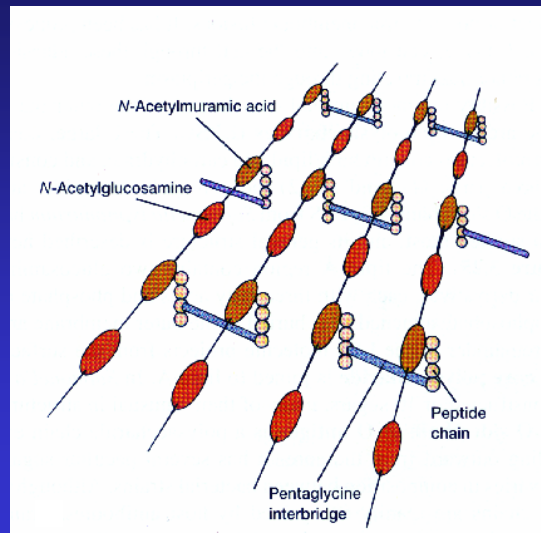


The Prototypic "Bug"



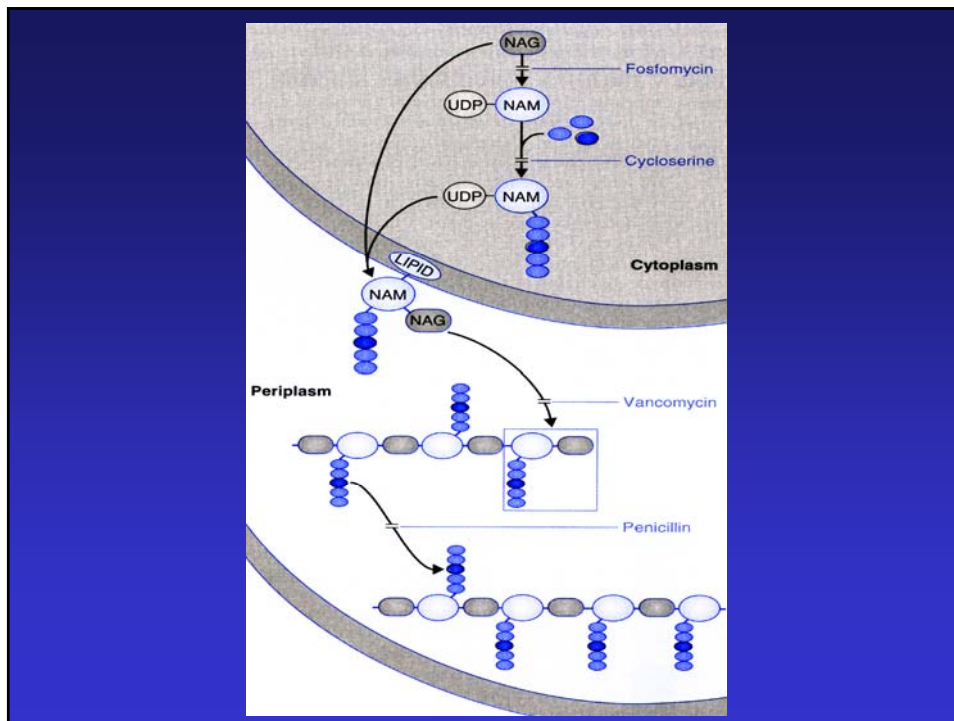


Bacterial Peptidoglycan

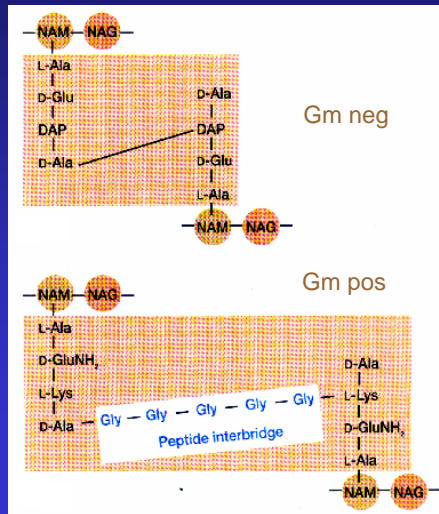


Peptidoglycan Biosynthesis

- Synthesis of water soluble, nucleotide-linked precursor in the cytoplasm
- Transfer of precursors from nucleotide to the bactoprenol membrane lipid (pivot), followed by the addition of substituents
- Addition of the prefabricated block to a linear glycan chain on the far side of the membrane
- Cross-linking to an adjacent chain via transpeptidation (enzymes are called penicillin-binding proteins)



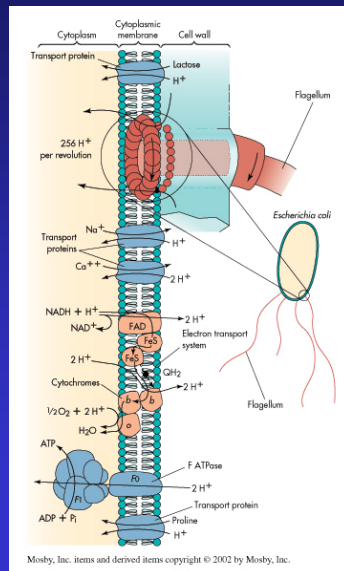
Transpeptidation Reaction



Final step in cell wall assembly is cross-linking of glycan chains




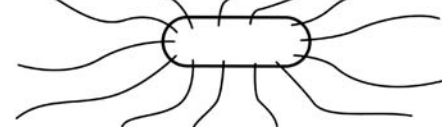
- Catalyzed by transpeptidase enzyme (PBPs)
- D-Ala-D-Ala required (terminal D-Ala released)
- Target for penicillin antibiotics

Cytoplasmic Membrane



- Phospholipid bilayer - some differences between Gm+/-
- Fulfills many of the functions of eukaryote organelles
- Production of ATP
- Energy for flagella
- Transport proteins
- Numerous biosynthetic processes

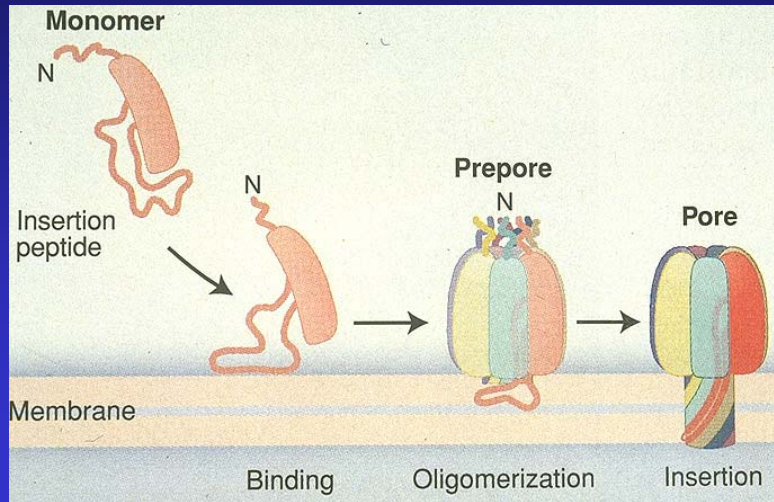
Flagella

Structure	Flagella Type	Example
	Monotrichous	<i>Vibrio cholerae</i>
	Lophotrichous	<i>Bartonella bacilliformis</i>
	Amphitrichous	<i>Spirillum serpens</i>
	Peritrichous	<i>Escherichia coli</i>

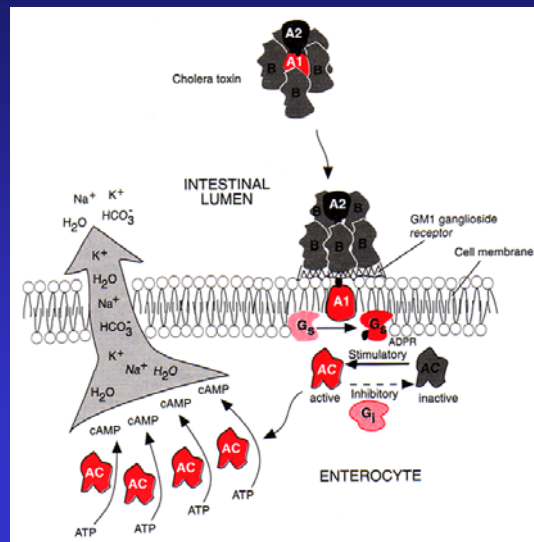
Secreted Products of Bacteria

- Exotoxins (distinct from endotoxin) are secreted bacterial proteins that are divided into 3 types:
 - A-B toxins (cholera, tetanus toxins)
 - Membrane disrupting toxins (hemolysins, α toxin)
 - Superantigens (toxic shock syndrome)
- Hydrolytic enzymes are bacterial products such as hyaluronidases and proteases that can degrade extracellular matrix and provide nutrients for the pathogen

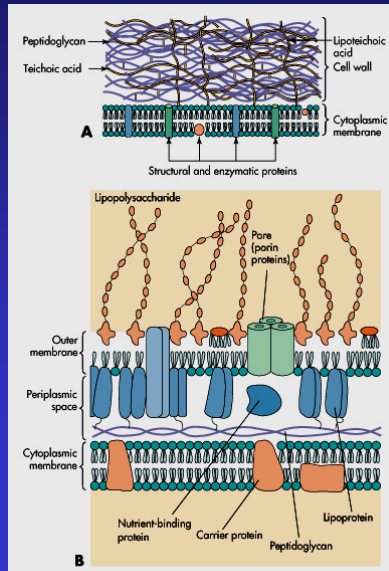
Alpha Toxin



Cholera A-B Exotoxin



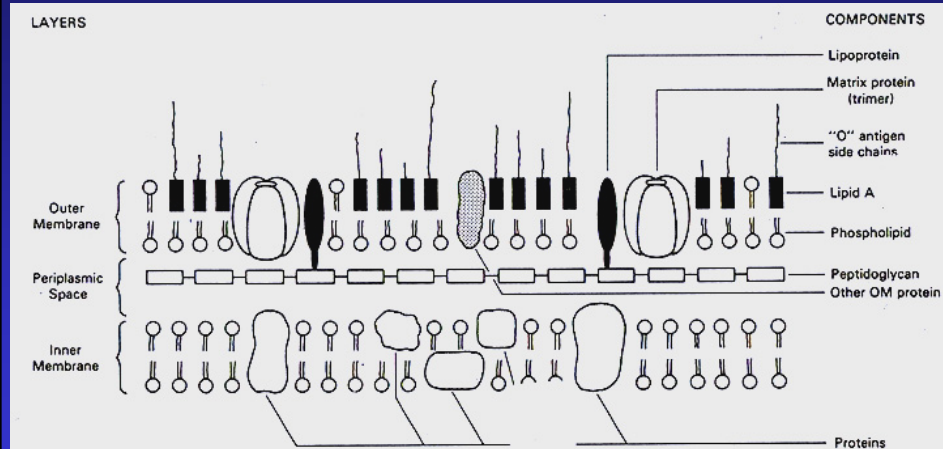
Differences Between Gram Positive and Gram Negative Bacteria



Spore Stain



Gram Negative Envelope



Endotoxin - Lipid A

- Essential for Gram negative bacterial survival and replication
 - Creates a permeability barrier for bacteria
- The entire complex lipid A structure is necessary for full activity
- Binds LPS binding protein in circulation, attaches to CD14 (receptor) on macrophage
- Triggers cytokine pathway by activation and direct binding of Toll receptor 4

When we sense lipopolysaccharide, we are likely to turn on every defense at our disposal; we will bomb, defoliate, blockade, seal off, and destroy all the tissues in the area..... All this seems unnecessary, panic-driven. There is nothing intrinsically poisonous about endotoxin, but it must look awful, or feel awful, when sensed by cells.

*Lewis Thomas
Germs, 1974*

So What Do I Really Need to Know?

- Application of phenotypic and genotypic classification systems
 - Gram stain - how it works
- Environmental reservoirs of bacteria
- Basic components of bacteria and their role in pathogenesis
 - Peptidoglycan synthesis
- Distinction between Gram positive and negative bacteria
 - The structure/role of endotoxin