Microbiology and Infectious Diseases
Lecture I: Bacterial Classification, Structure and Function, Part I
August 31, 2004
Lecturer: Dr. Lowy
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Some notes….
Although I did not have a tape for use in transcribing this lecture, it seems to be pretty consistent from skimming transcripts of the previous two years. When using old transcripts be conscious that the lecture was split up into two parts this year. Since the transcripts from 02 and 03 are in basically the same and are in outline format, I decided to use a text format for the use of those more inclined to learning in this way.

The major objectives of this lecture were to introduce us to microbiology, to explore the different bacterial classification systems, both phenotypic and genotypic, and to begin learning terminology for describing medically important pathogens.

The first few slides demonstrate the significance of learning about microbiology and infectious diseases in our medical training. In 1998, 13.3 million, or 25% of deaths worldwide were due to infectious diseases, not including cancers, respiratory, and cardiovascular deaths caused by infection. In lower income countries, infectious diseases are the leading cause of death. Acute respiratory infections and diarrheal diseases are the leading causes of death in children under five years old. Additionally, infectious diseases contribute to a dramatic loss of "life years" and morbidity.

It is important that there be a standard for classification of organisms so that there is a universal way to identify pathogens. How are bacteria classified? Phenotypic methods include distinguishing them based on gram staining properties, morphology, growth requirements, and biochemical properties. They can also be classified based on environmental reservoirs and their modes of transmission. Finally, genotypic methods of classification include 16s rRNA analysis and strain classification.

The gram stain is a very useful tool for examining bacterial morphology. The stain distinguishes bacteria based on differences in peptidoglycan thickness. First, a crystal violet stain is applied to the slide. Next, Gram's iodine stain is applied.~~, causing crystal formation of the violet stain.~~ Next, alcohol is applied to wash out the Crystal violet-gram's iodine complex. In cells with a thick peptidoglycan (gram +) the complex does not wash out because it is retained in the thicker peptidoglycan of the gram positive bacteria. In cells with little peptidoglycan (gram -), the crystal violet-gram's iodine complex is washed out**.** In the last step, a Safranin red counter stain is applied and the gram negative cells are stained red, but the gram +'s retain their purple/blue color.

We saw examples of gram staining in the next slides. The first of the example slides (#10) is a slide of pus containing **gram + streptococci** in chains (cocci look like spheres) and gram negative neutrophils. Slide # 11 contains **gram + staphylococci** (cocci in clusters). Slide #12 is shows **gram negative bacilli** (rods) exhibiting bipolar staining– the ends are more intensely stained than the centers. The next slide (#13) shows **gram negative staining diplococci**. You guessed it..groups of two cocci. These are characteristic of *Neisseria* which are responsible for gonorrhea. The last gram stained slide (#14) has intensely **gram positive long tubular organisms (pseudomycelia**) that

are larger than what we've seen previously. This is not bacteria, but a fungus of the *Candida* species.

Some bacteria do not stain well with the gram stain, and must be visualized in other ways. For example, **dark field microscopy** is used for *Treponema Pallidum* (responsible for Syphilis) and the **acid fast stain** is used for the mycobacterium responsible for tuberculosis. This is the one that you'll have trouble seeing if you're color blind.

Another way of classifying bacteria is on the basis of oxygen growth requirements. The slide he used to describe this showed an agar gel with lots of oxygen at the top and very little oxygen at the bottom of the gel. Each of the four categories that he mentioned was shown at the place in the gel where it would likely exist, based on its oxygen requirements. **Facultatively anaerobic** organisms (*Escherichia*) can grow in any oxygen conditions (anywhere in the gel). **Aerobic** organisms (*Pseudomonas*) would only grow at the top of the gel where oxygen is abundant. **Anaerobic** organisms (*Clostridia*) would grow at the bottom of the gel, where there is little oxygen. Finally, **Microaerophilic** organisms (*Campylobacteria*) need other nutrients, like carbon dioxide and modest oxygen to exist.

Dr. Lowy next briefly went over the charts for gram positive and gram negative and poorly staining bacteria. We're not responsible for them yet, but we will be. As far as gram negative bacteria, we will pay more attention to the rods, especially lactose fermenting and nonlactose fermenting bacteria of the aerobic and facultatively anaerobic category.

Bacteria can also be classified based on their environmental reservoirs. There are **endogenous bacteria** (ex: colon - anaerobic bacteroides; oropharynx - streptococcus; and skin - staphylococcus) that are part of our normal flora. These can play a role in protection against other microorganisms. **Exogenous bacteria** can exist in <u>water</u> (*cholera and Legionella*), in <u>food</u> (salmonella), in <u>ticks</u> (Lyme disease) and in <u>air and fomites</u> (*tuberculosis, bactillus anthracis*). **Fomites are infectious particles that, because of their small size, can be distributed over large areas. The fomites of mycobacteria tuberculosis are one example.**

There are some sites in the body that are normally sterile. These include the bloodstream, the CNS (especially CSF), the bladder, the lower respiratory tract, and the sinuses.

Cases:

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

- First, we think acute inflammation because of an infection. Where is the likely source of the bacteria causing the infection? It's either the skin or the catheter itself. We exam the pus and we see staphylococci (gram + cocci in clusters), which we know is endogenous for the skin. Next we do a series of biochemical tests to identify the species as *S. aureus.*

2. A 29 year old Peace Corp volunteer returns from his stint working in a remote village in Brazil. 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 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.

- This infection is likely the result of an exogenous bacterial agent. We take a sample of stool and find that the bacteria are gram negative rods. We plate it on agar and do a series of test to determine that it is non-lactose fermenting *shigella*.

Finally, we come to the genotypic classification methods of bacteria. Ribosomal RNA is used for this classification for 4 reasons: 1) it is present in all living cells 2) it has a highly conserved function 3) mutations are slow and constant and 4) there are highly conserved and highly variable regions that can be used as primers. 16s rRNA has been used to classify organisms into a three part phylogenetic tree: bacteria, archae, and eukarya families.

The 16s rRNA method with PCR allows for the speciating of not cultivatable bacteria (> 99% of bacteria). This method was used to classify the bacteria causing Whipple's disease because it was found to be similar to the actinomycetes family. Narrowing it down to this level gave scientists an idea of what type of media the bacteria could be grown on and what type of antibiotics it might be susceptible to.

Sometimes, it is necessary to determine whether bacteria leading to the same infection come from the same strain. Pulse Field Gel Electrophoresis is used for molecular subtyping to determine whether strains are identical. DNA is cut by restriction enzymes, and banding profiles are compared.