### **BIOL215: MICROBIOLOGY FOR HEALTHCARE PROFESSIONALS**

# Lecture notes for Exam 1

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# **INTRODUCTION:**

Microbiology – the study of microbes/ microorganisms

Microbes - living organisms too small to see without magnification

Microscope – invented by Leeuwenhoek (1600s)

Before we begin discussing microbial species:

Binomial Nomenclature	naming of organisms as Genus species
	developed by <b>Linnaeus</b> (1700s)

Ex. <b>Escherichia coli</b> (E. coli)	<ul> <li>bacterium in GI tract</li> </ul>
	- named in honor of Escherich (guy who discovered it)

# **MICROBES INCLUDE:**

BACTERIA	- prokaryotes (no nucleus) - unicellular - ex. <b>E. coli, Staphylococcus aureus</b> (MRSA: Methicillin-resistant Staphylococcus aureus)
PROTISTS	-eukaryotes (nucleus) - unicellular - ex. <b>Naegleria fowleri</b> (brain-eating amoeba) – in warm freshwater, enters nose/sinuses
HELMINTHS	- eukaryotes - multicellular 'worms' - ex. <b>Dracunculus medinensis</b> (Guinea worm) – in freshwater in Africa, nearly eradicated - basis for medical logo, the <b>Rod of Asclepias</b>

FUNGI	- eukaryotes
	- unicellular or multicellular
	- ex. <i>Candida albicans</i> (yeast infection)
VIRUSES	- not prokaryote or eukaryote; not alive!
	- protein coat surrounding DNA or RNA
	- ex. Influenza (the flu) – note: no Genus or species name
PRIONS	- not prokaryote or eukaryote; not alive!
	- infectious proteins
	<ul> <li>- ex. Mad Cow Disease (BSE – Bovine Spongiform Encephalopathy) in cows</li> </ul>
	- ex. CJD - Creutzfeldt-Jakob Disease in people, also called 'KURU" in New Guinea
	- ex. CWD – Chronic Wasting Disease in deer, elk
	- ex. <b>scrapie</b> in sheep, goats

Back to the textbook definition of microbiology:

- \* some microbes are **not alive** (ex. viruses, prions)
- \* some microbes can be seen with naked eye (ex. helminths)

### **BENEFICIAL MICROBES:**

Most microbes are not pathogenic (disease-causing)

Many microbes are helpful, for example:

#### 1. Vaginal Bacteria

- "MICROBIOME" naturally-occurring bacteria in and on the body
- ex. Lactobacillus spp. (such as L. acidophilus and other species)
- acid-producing (lactic acid)
- acid-loving (acidophilic)
- pH of normal vagina = 4
- inhibit the growth of pathogenic microbes by **competitive exclusion/microbial antagonism**
- Candida albicans (fungus that causes yeast infection) growth is inhibited at pH < 4
- Gardnerella vaginalis (bacteria that cause BV, bacterial vaginitis) is inhibited at pH < 4
- when Lactobacillus is absent (after antibiotics, for example), vaginitis is thus more likely

### 2. Fecal Transplantation

- use of a donor's healthy feces (with its microbiome) to treat a patient with CDIFF
- CDIFF is Clostridium difficile (bacteria) that cause gastrointestinal infection
- causes almost 30,000 deaths per year in USA
- most common in hospitalized patients and in nursing homes
- success rate with fecal transplant is 94% (three times better than with antibiotics)

#### **3. Food Production**

- bread, wine, beer made with live yeast (fungus) Saccharomyces cerevisiae
- yogurt made with live bacteria Lactobacillus bulgaricus and other bacteria
- cheese made with live bacteria (many different species)
- etc...

# 4. Probiotics

- live microbes eaten for better health (not scientifically proven, however)
- for GI problems, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD)

\* The rest of the semester will cover pathogenic microbes

#### **HISTORICAL FIGURES:**

Leeuwenhoek (1600s) - first microscope

Jenner (1700s) – first vaccine (immunization, inoculation)

- inoculated people with **cowpox** virus to prevent **smallpox** virus
- cowpox is mild and not lethal, causes skin rash (milkmaid's hands, from milking cows)
- smallpox is virulent and lethal
- term "vaccine" derived from "vacca" = cow in Latin

Pasteur (1800s) - developed pasteurization (use of heat to reduce bacteria in food/drink)

- pasteurization prolongs shelf life and makes safer foods/drinks
- originally used to prevent wine from turning sour
- Acetobacter bacteria convert alcohol into acetic acid (vinegar; vin aigre...sour wine)
- also disproved spontaneous generation idea that organisms can arise from nothing
- used gooseneck flasks and broth
- also demonstrated biogenesis idea that live cells only come from live cells
- also developed aseptic techniques sterile equipment used in research and healthcare

Semmelweis (1800s) - implemented handwashing in hospitals to reduce spread of infection

- doctors washed hands with a chlorine solution (similar to bleach)
- infection rates dropped from 10% to 1%
- still today, hospital-acquired (healthcare-associated, nosocomial) infections
- 30% of ICU patients have nosocomial infections!

Lister (1800s) - first use of an antimicrobial chemical (phenol) during surgery to prevent infection

<b>Koch</b> (1800s)	<ul> <li>first to prove a species of microbe can cause a specific disease</li> </ul>
	- <b>anthrax</b> (caused by <b>Bacillus anthracis</b> bacteria), respiratory disease, in mice
	- Koch's Postulates:
	- isolate in culture the suspected microbe from diseased host
	- administer the microbe to healthy new subject
	- observe disease progression
	<ul> <li>isolate same microbe from new host, now with same disease</li> </ul>
Fleming (1900s)	- discovered first <b>antibiotic</b> (chemical taken internally that kills bacteria)
	- penicillin, derived from fungus Penicillium notatum
	- the fungus was exhibiting <b>competitive exclusion</b> to fight bacteria
Marshall (1090a	) first to prove <b>perticulates</b> can be caused by besteria
warshall (1980s	) - first to prove <b>peptic uicers</b> can be caused by bacteria
	- peptic ucers are <b>resions</b> (open sores) in stomach
	- Helicobacter pylori (acid-tolerant bacteria in stomach)
	<ul> <li>he used Koch's Postulates on himself!</li> </ul>
	- he gave himself the bacteria, developed ulcers, then treated with antibiotics

Peptic ulcer diagnosis:

1. **symptoms** (subjective; reported by patient) and **signs** (objective; can be observed or measured)

- dull pain in the stomach, heartburn in chest symptom
- weight loss (not wanting to eat because of pain) sign
- nausea or vomiting (vomit looks like coffee grounds or is bloody) sign
- anemia (low blood cell count; tired, short of breath, pale skin) signs and symptoms
- dark, tarry stool sign

# 2. EGD - esophagogastroduodenoscopy (endoscopy)

- examine stomach lining for ulcers (open sores)

# 3. Urea Breath Test

- patient swallows isotope-labelled urea
- urea = (NH<sub>2</sub>)<sub>2</sub>CO
- labelled urea has either <sup>13</sup>C or <sup>14</sup>C (normal is <sup>12</sup>C)
- isotopes have different numbers of neutrons
- H. pylori bacteria convert urea into ammonia and CO2, via enzyme urease



- exhaled breath is analyzed for  ${}^{13}C$  or  ${}^{14}C$  (in  $CO_2$ )
- positive result if the isotope is present in breath

Peptic ulcer treatment (all of the following):

Antibiotics	<ul> <li>two antibiotics taken</li> </ul>
	- ex. Metronidazole ("Flagyl")
	- ex. <b>Clarithromycin</b> ("Biaxin")
Acid secretion inhibitor	- reduces acid production
	- ex. <b>Omeprazole</b> ("Prilosec")
Antacid	- buffers acidity
	- ex. Bismuth subsalicylate ("Pepto-Bismol")

# **MICROBES IN THE LAB:**

How small are microbes?

Measured in micrometers (microns) - µm

1000 µm / 1 mm (millimeter)

Sometimes measured in **nanometers** - nm

1000 nm / 1 μm

FUNGI and PROTISTS	>	BACTERIA	>	VIRUSES	
4 – 40 μm		0.1 – 10 μm		0.03 – 0.3 μm	

Q. How many  $\mu m$  long is a helminth that is 10.5 mm long?

<u>10.5 mm</u>	х	<u>1000 μm</u>	=	10,500 μm
1		1 mm		

### Q. How many nm long is a virus that is 0.05 $\mu m$ long?

<u>0.05 μm</u>	х	<u>1000 nm</u>	=	50 nm
1		1 µm		

#### **Staining Microbes:**

Staining is necessary to see microbes under the microscope (otherwise are colorless)

- 1. Simple stain a single dye is used
- 2. Differential stain uses two or more dyes

- can discern between types of bacteria

#### Ex. Gram stain

- Gram-positive bacteria = purple
- Gram-negative bacteria = pink/red

How does the Gram stain work?

Gram-positive Crystal violet (purple dye) Looks purple Crystals form, looks purple Cell wall unaffected by alcohol peptidoglycan outer cell wall

Looks purple

# Gram-negative





Crystals form, looks purple



Cell wall partly dissolved by alcohol

Crystals escape from cell

LPS outer cell layer (lipopolysaccharide)

lipid-based

LPS only in Gram-negative



Safranin (pink dye)

Iodine

Alcohol

# 3. Negative stain colors background dark, bacteria appear colorless/white used for bacteria that repel stains also allows visualization of capsules = protective 'shell' around some bacteria that resist the immune system and antibacterial drugs ex. Streptococcus pneumoniae is pathogenic when it has capsules, but not pathogenic when it lacks capsules (immune system can fight off nonencapsulated strains)

\* strains are different varieties of one species of bacteria, ex. encapsulated and nonencapsulated strains of *S. pneumoniae* 

4. Flagellar stain	<ul> <li>allows visualization of flagella = whip-like structures used for motility</li> <li>motility = ability of some bacteria to move in fluids</li> </ul>			
	- atrichous = no flagella			
	<ul> <li>monotrichous = one flagellum</li> </ul>			
	<ul> <li>amphitrichous = two flagella; one at each end of cell</li> </ul>			
	<ul> <li>- lophotrichous = tuft of flagella at one end</li> </ul>			
	<ul> <li>peritrichous = covered in flagella</li> </ul>			
5. Acid-fast stain	- rapid diagnostic stain to test for <b>tuberculosis</b> and <b>leprosy</b>			
5. Acia iust stain	- positive result (red bacteria) will only be either:			
	- Mycobacterium tuberculosis = bacteria that causes tuberculosis (TB)			
	- respiratory disease			
	- highly contagious			
	<ul> <li>many strains are resistant to antibiotics,</li> </ul>			
	ex. MDR TB (multidrug resistant TB)			
	XDR TB (extensively drug resistant TB)			
	<ul> <li>Mycobacterium leprae = bacteria that causes leprosy ("Hansen's disease")</li> </ul>			
	<ul> <li>skin disease, open sores (lesions) on cool extremities</li> </ul>			
	<ul> <li>extremities = face, fingers, toes</li> </ul>			

- -contagious
- can be treated with antibiotics
- grown on armadillos for research (low body temp)

### \* side note: what is the TB test?

#### TST = tuberculin skin test

- tuberculosis bacterial protein (tuberculin) injected into the skin
- 48-72 hours later, the induration (welt) is measured
- 6-10 mm OK, but more than that means the person is vulnerable to TB

# 6. Endospore stain - allows visualization of bacterial endospores

- endospores = highly resistant to chemicals, heat, radiation, etc...
- seed-like, dormant stage of some bacteria
- Bacillus species and Clostridium species, including:
- ex. Bacillus anthracis (causes anthrax; respiratory disease -- mentioned earlier)
- ex. Clostridium difficile (causes Cdiff -- mentioned earlier)
- ex. *Clostridium botulinum* -- causes **botulism** = muscle paralysis
  - type of food poisoning
  - can cause **Infant hypotonia** (**floppy baby syndrome**) when babies eat *C. botulinum* contaminated honey
- ex. *Clostridium tetani* causes tetanus = uncontrolled muscle contraction
   endospores enter body via puncture wound (step on rusty nail)