

Microbiology Final

I. Background Info

A. sterilization

1. absolute- gets rid of all bac in an area usually by physical means

B. disinfection

1. removes only infectious organisms- those that cause disease- usually by chemical means

C. antiseptic

1. disinfectant that removes bac on the surface of the skin

D. sanitization

1. use hot water and soap which reduces bac count, but not disinfection

II. Sterilization

A. removal

1. filtration- holds back bac, lets fluid through

- a. in lab or clinic, use filter made out of nitrocellulose w/ pores that are .22 micron

1. removes bac since bac are 1 micron

2. use this method when want to sterilize heat labile liquid, ex: serum

A. many sterilization methods involve heat which destroy antibodies in serum, so use filtration- serum w/ antibodies goes through, bac don't

- b. sterilize water supply by passing water through 5 feet of sand

1. water goes through, bac stays behind

B. killing

1. heat

a. moist heat

1. heating

A. raise temp to b/t 60-65 degrees Celsius- kills most bac

B. ex: pasteurization- 62 degrees for 30 min

1. does not kill proteins in milk, but inhibits bac growth

2. boiling

A. 100 degrees Celsius- will kill vegetative cells, spores, and thermophiles

B. worried about spores b/c can land on surgical instruments

3. live steam (better than boiling)

A. takes energy to break water molecules into vapor (heat of vaporization)

B. when steam lands on bac cell, it condenses into water, releasing energy

C. this energy is used to kill bac

4. steam under pressure

A. use autoclave- pressure cooker used in lab

B. causes water to go higher than 100 degrees which can kill spores better

C. 15 lbs of pressure = 121 degrees- takes only 15-20 mins to kill bac (spores)

b. dry heat

1. incineration- burn it in fire

A. 2 ex:

1. patient in hospital- full of pus and sores- burn sheets and bandages

2. lab- flame loop w/ dry heat to sterilize it

2. oven

A. 180 degrees Celsius for 2 hrs

B. make sure containers can tolerate heat- ex: glass and metal

C. use for things that won't work w/ autoclave- ex: powders and gels

2. radiation

a. ultraviolet light

1. used in germicidal lamps
2. used for air-borne pathogens (doesn't work for glass or solutions)
3. ex: operating room- needs to be sterile
 - A. after surgery, turn off regular lights and turn on UV lamps
 - B. UV light causes thymine dimers which stops transcription and kills bac
 1. can't leave UV on all the time b/c lethal to patients, too
 2. turn off reg lights b/c don't want photoreactivation to repair the DNA (photolyase removes dimers in the presence of light)

b. x-rays

1. direct method
 - A. form single-strand breaks in DNA
2. indirect method
 - A. works on medium, forming reactive oxygen species (ROS) which kill bac

III. Disinfection

A. agents that damage cell membranes (cause leakage of molecules—>lysis of cells- kill cells)

1. cationic agents (positively charged molecules)

a. quaternary ammonium salts- have 2 parts:

1. a long strand of C and H = non-polar lipid portion that fits into membrane
2. a charged polar part of molecule that messes up the membrane

2. anionic agents (negatively charged molecules)

a. detergents

1. composed of soap w/ molecule that has neg charge
2. fatty acid (soap) part gets into membrane and charged part messes it up

3. phenolic compounds (used to use phenol itself, but it killed bac and patients)

a. alkyl phenols = cresols

1. alkyl= methyl or ethyl group
2. cresols together w/ soap = lysol

b. diphenyl compounds- halogenated (F, Cl, Br, and I)

1. ex: hexachlorophene- used for 2 purposes:

- A. germicidal soap- surgeons can wash hands w/ it b/f surgery
- B. antiperspirant- not used so much anymore since discovered it's neurotoxic- bad for human nervous system

4. alcohols

1. ethanol- skin disinfectant (swab skin b/f injecting a needle)

B. agents that denature proteins

1. acids and alkalis

a. strong acids and bases- dissociated, have pH effect- denature proteins

b. weak acids- proton stays on acid, but that's also a toxic molecule

1. ex: benzoic acid- inhibits bac growth by affecting proteins- prevents food spoilage

2. solvents

- a. ex: acetone- will denature proteins and inhibit bac growth

C. agents that modify functional groups in proteins or DNA

1. heavy metals (modify proteins)

a. ex: mercury, arsenic, and silver bind to SH (sulfhydryl group)

1. cell needs S which is in certain amino acids (cysteine, methionine)
2. S needed for structure and function of proteins

b. this binding messes up bac proteins, inhibiting bac growth

c. ex: of heavy metals:

1. organic mercurials (not mercury itself b/c it kills patients)
 - A. mercurochrome = antiseptic
 2. silver compounds
 - A. silver nitrate
 1. 1% of this solution is put in babies' eyes when they're born
 2. it targets gonococcus which cause venereal disease gonorrhea
 3. afraid mother has gonorrhea and baby will get it when passes through birth canal- it targets baby's eyes
 - B. silver sulfadiazene = silvadene- used to treat 2 problems:
 1. burns
 - a. can cause infection b/c skin is gone and it's usually a barrier
 - b. silver sulfadiazene is antibiotic that prevents infection and drying out of skin
 2. decubitus ulcers = bedsores
 - a. a bed sore is skin breakdown in areas in contact w/ bed
 1. ex: calves, buttocks, shoulders...
2. oxidizing agents (modify proteins)
 - a. halogens
 1. skin disinfectants
 - A. iodine
 - B. betadine (used in clinical setting- swab over area b/f surgery)
 2. water disinfectant
 - A. chlorine (used in pools)
 - b. hydrogen peroxide
 1. used to sterilize surgical devices and contact lenses
 3. alkylating agents (modify bases in nucleic acids in DNA)
 - a. formaldehyde
 1. preserves fresh tissue
 - A. keeps biopsy tissue from decomposing b/f reaches lab b/c kills bac in tissue
 2. gas sterilization of rooms
 - A. use gaseous formaldehyde to disinfect hospital room of coughing patient
 - b. glutaraldehyde
 1. cold sterilization of instruments
 2. first have to remove blood and guts through ultrasonic soundwaves
 - c. ethylene oxide
 1. gas sterilization of things you can't put in autoclave like electronic equipment

IV. Antimicrobial Therapy

A. need to consider the interactions of drug, microbe, and host b/f prescribing meds

B. 6 ways these factors interact:

1. microbe vs. host- mechanisms by which bac can cause disease

a. 3 mechanisms:

1. invasiveness

A. the ability of bac to penetrate skin and mucous membranes (barriers) of body and colonize the target tissue (settle, grow, and multiply)

2. toxin production

A. tetanus- neurotoxin that causes spastic paralysis

B. botulinum- neurotoxin that causes flaccid paralysis

C. diphtheria

3. enzymes

A. exoenzymes digest tissue

- b. need to know mechanism by which disease came about
 - 1. if through toxin, use antitoxin first- not just antibiotic
 - 2. w/ infant meningitis, use broad-spectrum antibiotics b/c no time to find best meds
- 2. host vs. microbe- how host defends itself
 - a. innate immunity- always there ready to go, nonspecific
 - 1. physical
 - A. barriers
 - 1. skin- prevents bac from entering body
 - 2. mucous membranes- protect body cavities so bac don't enter underlying tissue
 - B. washing effects
 - 1. tears and saliva wash bac away
 - C. expulsive effects
 - 1. coughing and sneezing- blow bac out
 - 2. ciliary action- sweeps mucous up in respiratory tract
 - 2. chemical
 - A. acidity of stomach (HCl) kills bac (like those bac from contaminated food)
 - B. lysozymes in tears- enzymes that destroy cell wall of bac
 - 3. cellular
 - A. phagocytosis- WBC in body engulf bac and destroy them
 - b. adaptive immunity- only comes in response to infection, highly specific to that infection
 - 1. referring to antibodies- spec in response to an org, don't protect against a dif org
 - 2. 4 dif types of antibodies:
 - A. antitoxins- neutralize bac toxins
 - B. lysins- cause bac cells to lyse
 - C. agglutinating- cause bac to clump (makes it easier for phagocytosis)
 - D. opsonizing- enhance phagocytosis
 - 1. putting these antibodies on cell makes phagocytosis work better
- 3. drug vs. microbe- ways antibiotics target bac
 - a. there are a number of different targets for antibiotics- some...
 - 1. inhibit cell wall synthesis
 - 2. damage cell membrane structure
 - 3. inhibit DNA, RNA, or protein synthesis
 - 4. inhibit synthesis of small molecules in cell- vitamins, precursors...
 - b. if org is resistant to drug that acts on a particular target, it's likely to be resistant to another drug that attacks the same target, so switch to a drug that works on a dif target
- 4. microbe vs. drug- mechanisms of bac resistance
 - a. drug can't get in or stay in
 - 1. change in permeability
 - A. drug needs to get into cell via a transport system
 - B. if bac cell has a mutation in gene to make transport protein, drug can't be brought into cell
 - 2. efflux pumps- as fast as bac gets in, cell pumps it out
 - b. change in cellular metabolism-
 - 1. talking about antibiotics that act as competitive inhibitors for some part of metabolism in cell- messes up enzyme so bac can't do metabolism
 - 2. s/t changes in bac cell metabolism cause antibiotics to have less effect:
 - A. bac cell makes more substrate->less likely antibiotics will block active sites
 - B. bac uses alternate pathway to make the product, so cell can still grow w/o using pathway that can be blocked by antibiotic

- c. affects drug molecule itself- 2 dif ways:
 - 1. bac enzyme breaks down the drug
 - A. ex: penicillase breaks down penicillin
 - 2. bac enzyme inactivates the drug- modifies it in some way
 - A. ex: by acetylation or phosphorylation- adding a/t group inactivates the drug
 - B. this mechanism is usually done by plasmid enzymes
 - 1. gene on plasmid DNA codes for enzyme that will modify antibiotics so antibiotics doesn't work- makes bac resistant (transferred by conjugation)
- d. change in target site
 - 1. ex: if have drug that inhibits protein synthesis by binding to a certain part of a ribosome (ex: 30 s subunit), but mutation causes change in ribosomal protein, so drug can't bind, then bac is resistant to that drug
- 5. drug vs. host- how drugs affect host (side effects)
 - a. want drug w/ selective toxicity- affects bac/bug, not the host- but drugs can have side effects:
 - 1. allergic reactions
 - A. immediate- anaphylactic shock
 - 1. can treat patient w/ adrenaline/epinephrine to stop this
 - B. delayed- rashes
 - 2. toxicity
 - A. renal toxicity- damages kidneys
 - B. hepatic toxicity- damages liver
 - C. nervous system toxicity- damages nervous system
 - 3. secondary effects
 - A. can suppress normal flora, so less nutrient competition for pathogens
 - B. this causes outgrowth of pathogens- ex: Clostridium difficile which causes enterocolitis
- 6. host vs drug- ways host could prevent drug from working
 - a. gastric acidity
 - 1. stomach acid destroys antibiotics b/f it gets to bac, so need to inject drug instead
 - b. antibiotics inhibits growing cells only
 - 1. so if have abcess= rotting tissue- bac cells not growing , so antibiotics won't work
 - 2. ex: penicillin and streptomycin only work on growing cells
 - c. necrosis=tissue destruction
 - 1. capillary network is destroyed, so drug can't be circulated to infection site to kill bac
 - d. dose vs available (host factors)
 - 1. when giving patient a dose of antibiotics, have to consider 4 factors:
 - A. absorption- only some of the drug can get through tissue from intestine to bloodstream, so less gets absorbed in the bloodstream
 - B. protein binding- proteins in blood act as sponges- take antib. out of circulation
 - 1. albumins are the proteins that bind the drugs
 - C. detoxification- liver enzymes inactivate the drug
 - D. excretion- some of the drug passes out in urine
 - 2. of 100 mg given to patient, only some absorbed into bloodstream- the rest is detoxified, bound w/ proteins, or excreted, so less is available to patient

V. Difference Between Antimicrobial Agents and Antibiotics

- A. antimicrobial agents- general, refers even to drugs that are chemically synthesized (but includes antibiotics)
- B. antibiotics- specific, refers to drugs of microbial origin (bacteria or fungus)
 - a. there are microorganisms produce chem that kill bac- ex:
 - 1. the mold penicillium produces penicillin
 - 2. the mold streptomyces produces streptomycin
 - 3. the bac Bacillus produces bacetracin

VI. Properties of Drugs

A. bacteristatic vs. bactericidal

1. bacteristatic drugs- inhibit bac growth and are reversible
 - a. don't kill bac and when take drug away, bac starts growing again
2. bactericidal drugs- lethal (kill cells) and are irreversible

B. spectrum

1. narrow spectrum- affect 1 org or a few org
2. broad spectrum- affect a large number of org (G+, G-...)

C. therapeutic index

1. it's the ratio of toxic dose to curative dose
 - a. dose- amount you give patient
 - b. toxic dose- the amount of drug which will be harmful to patient
 - c. curative dose- the amount of drug which will get rid of bac
2. for a good antibiotic, need high therapeutic index
 - a. if index is one, the same amt that will kill bac will kill patient
 - b. want it to take a little to cure infection and a lot more to be harmful to patient
 1. so want index to be 10, 20, 100, or more
 - c. therefore, if patient takes too much of a drug, it won't harm the patient

VII. Cell Wall Synthesis

A. takes place in 3 locations:

1. inside cytoplasm (in cell)
 - a. starts with UDP-N-Acetyl muramic acid (NAM)
 1. UDP = uridine diphosphate
 2. many biosynthetic reactions need high energy mol for reaction to take place
 - b. 3 amino acids are added to NAM:
 1. L-Alanine
 2. D-Glutamic acid
 3. L-Lysine
 - c. in separate reaction, L-Alanine becomes D-Alanine in the cytoplasm
 1. w/ the help of the enzyme racemase
 - d. 2 D-alanine's are connected together forming the dipeptide D-Alanine-D-Alanine
 1. w/ the help of the enzyme synthetase
 - e. D-Alanine-D-Alanine attaches to lysine of previous 3 amino acids, forming a pentapeptide
 - f. the bond between the 2 phosphates in UDP breaks, forming UMP and P-NAM-pentapeptide
 - g. UMP (U-P) stays in the cytoplasm
2. inside cell membrane
 - a. P-NAM with pentapeptide enters the cell membrane
 - b. a carrier lipid with one phosphate (carrier lipid-P) attaches to P-NAM
 1. attaches by pyrophosphate linkage- 2 phosphates connect carrier to NAM
 2. carrier lipid is undecaprenyl which means 11 prenyls
 - a. prenyl has 5 carbons, so carrier lipid is a 55 carbon chain
 - c. NAG is added to NAM
 - d. in *S. aureus*, glutamic acid is converted to glutamine by the addition of an amine group- NH_2
 - e. bridging group is added to third amino acid of pentapeptide
 1. in *S. aureus*, bridging group is pentaglycine and it attaches to L-Lysine
 - f. the bond between P and NAM breaks, forming carrier-P-P
 - g. carrier-P-P remains in the cell
 - h. carrier-P-P splits into carrier-P and P via an enzymatic reaction
 1. carrier-P is now ready to start the process again

3. outside cell membrane

- a. pentapeptide-NAM-NAG goes outside the cell membrane
- b. then 2 imp processes take place:
 - 1. transglycosylation
 - A. addition of subunit to preexisting wall
 - B. this causes the growth of the cell
 - C. bond b/t adjacent NAM-NAG subunits is broken and more NAM-NAG is inserted to enlarge the backbone
 - D. this takes place many times until one cell becomes two
 - 2. transpeptidation
 - A. the process of cross-linkage- attaching bridging groups to D-Alanine (4th amino acid) of tetrapeptides of other chains
 - B. in the process, terminal D-Alanine of pentapeptide comes off, forming a tetrapeptide

VIII. The Organization of Antibiotics by their Target Sites

A. those that affect cell wall synthesis

1. B-lactam antibiotics

a. chemical structure is 2 rings- one ring is B-lactam which inhibits cell wall synthesis

b. 2 ex:

1. penicillins

- A. original penicillin (pen. G) is from penicillium notatum=a mold
- B. it inhibits transpeptidation
- C. bactericidal- kill by weakening cell wall—>lysis=cell death
- D. only kill growing cells
- E. problem of resistance- some bac produce the enzyme penicillinase which destroys penicillin
- F. penicillin has 1 part that could be substituted, forming derivatives- all dif:

Drug	Spectrum	Penicillinase	Acid	Activity
1. penicillin G (benzyl penicillin)	narrow	sensitive	labile (destroyed by stomach acid, so need to inject it)	high (curative dose is low- powerful drug)
2. methicillin	narrow	resistant	labile	1/30 of pen G
3. oxacillin	narrow	resistant	stable (not destroyed by stomach acid)	1/10 of pen G
4. ampicillin	broad	sensitive	stable	½ of pen G

G. there are is a way to get around penicillinase:

- 1. clavulanic acid inhibits penicillinase
- 2. so can take augmentin=amoxicillin+clavulanic acid
 - a. amoxicillin is similar to ampicillin
 - b. aug is broad spectrum, stable in acid and is protected from penicillinase b/c of clavulanic acid

H. penicillin spectrum is used to treat infections of G+ org- ex:

- 1. neisseria- causes gonorrhea
- 2. spirochaetes- cause syphilis

I. problem w/ allergic reaction to penicillin

- 1. take patient's history and have adrenaline in case of anaphylactic shock

2. cephalosporins

- A. have B-lactam ring, but have dif 2nd ring and dif parts of molecule than pen
- B. also inhibit transpeptidation (b/c B-lactam ring does that)
- C. cephalosporin is sensitive to penicillinase
- D. there are derivatives of cephalosporins: (can be sens or resist to penicillinase)
 - 1. cephalothin
 - 2. cephaloridine
 - 3. cefoxitin
 - 4. cefaclor

2. D-cycloserine

- a. inhibits L-Alanine to D-Alanine and 2 D-Ala's to D-Ala-D-Ala
- b. used for G+ and G- and particularly used to inhibit mycobacterium tuberculosis- acid-fast
 - 1. it's an antitubercular drug and not too many drugs are
- c. bac can become resistant due to:
 - 1. loss of transport- bac cell does not take up drug into cell
 - 2. altered target- bac enzymes (target of drug) don't bind the drug
- d. side effect of drug- causes central nervous system toxicity

3. bacitracin

- a. it's a polypeptide antibiotic (e/t else was a small molecule)
(new area of study: human body produces antibacterial polypeptides)
- b. inhibits step in cell wall synthesis when carrier lipid-P-P becomes carrier-P (dephosph of carrier lipid)
- c. side effect- nephrotoxic- causes kidney damage, so only used topically
- d. resistance of bac to bacitracin is rare

4. vancomycin

- a. inhibits transglycosylation
- b. side effect- it's toxic- causes damage to 8th cranial nerve (auditory), so may cause deafness
- c. use it as last resort b/c of toxicity, but there's an emerging problem of vancomycin resistance

B. those that affect cell membrane structure

1. some are antibacterial- ex:

- a. polymixins A, B, C, D, and E, especially poly B and poly E/colistin
 - 1. they change cell membrane structure, resulting in leakage, resulting in lysis
 - 2. used in the treatment of G- infections
 - 3. side effects: nephrotoxic (damages kidneys); therefore, only used topically
- b. gramicidin
 - 1. acts similarly to detergents- messes up membrane and causes problems

2. some are antifungal

- a. nystatin
 - 1. used for infections caused by candida albicans (yeast, eukaryotic)
 - A. causes yeast infections, like thrush in the mouth
- b. amphotericin B
 - 1. used for treatment for systemic fungal infections
 - 2. it's nephrotoxic
 - A. taken internally anyway b/c fungal infections are very bad and hard to treat

C. those that affect DNA synthesis

1. novobiocin

- a. inhibits the enzyme DNA gyrase
 - 1. it's the enzyme that help straighten out knots when unwinding DNA for replication
- b. spectrum: used for G+ infections- and for staph infections that are resistant to other drugs
- d. problem: toxicity- use it rarely

2. griseofulvin

- a. antifungal
- b. inhibits mitosis
- c. used in the treatment of dermatophytes- which attack hair, skin, and nails

D. those that affect RNA synthesis

1. rifampicin (rifampin)

- a. inhibits RNA polymerase
- b. it's used for G+ infections and to treat mycobacterium tuberculosis (antitubercular)
- c. resistance: enzyme (RNA pol) doesn't bind drug (target site is changed)

E. those that affect protein synthesis

1. those that affect the 30s subunit of the ribosome

a. aminoglycosides

1. one ex=streptomycin

- A. inhibits protein synthesis- binds to protein in 30s subunit
- B. causes misreading of the genetic code
 - 1. it causes structural changes in the reading frame of the ribosome
 - 2. this causes the wrong tRNA to bind, so the wrong aa are put together
- C. it's bactericidal - only affects growing cells
 - 1. one reason is messing up proteins destroys the cell mem b/c wrong prot are put in, causing lysis
- D. 2 mechanisms of resistance:
 - 1. plasmid has gene that codes for enzyme that phosph drug, inact. it
 - 2. change in target- a change in chr that makes rib prot causes rib to be unable to bind drug
- E. broad spectrum antibiotic- works against G+, G- and tuberculosis
- F. problem: toxic
 - 1. neurotoxicity, nephrotoxicity, damages 8th cranial nerve—>deafness

2. other aminoglycosides:

- A. neomycin
- B. kanamycin
- C. gentamycin

b. tetracyclines

1. prevent binding of tRNA to the 30s subunit of the ribosome

2. ex include:

- A. tetracycline
- B. chlortetracycline
- C. oxycycline
- D. minocycline

3. broad spectrum antibiotics

4. bacteriostatic- inhibit growth, don't kill bac cell

5. 2 mechanisms of resistance:

- A. changed permeability- drug can't be brought in by transport protein
- B. inactivation (plasmid enzymes inactivate drugs by phosph or acetyl)

6. side effect

- A. photosensitive reaction which results in tooth discoloration
 - 1. drug+sunshine—>black teeth
- B. toxic to liver and GI system

2. those that affect the 50s subunit of the ribosome

a. chloramphenicol

1. inhibits peptide bond formation (so aa can't connect into protein)
2. broad spectrum
3. bacteristatic
4. 2 mechanisms of resistance:
 - A. plasmid gene makes enzyme that will acetylate drug
 - B. change in gene of chr that makes rib prot, so 50s subunit doesn't bind drug
5. side effect- it's carcinogenic
 - A. but if it's the only drug that will work, give it and worry about cancer later

b. macrolides

1. ex include:
 - A. erythromycin
 - B. oleandomycin
 - C. carbomycin
2. inhibit peptide bond formation
3. not as toxic as chloramphenicol- not carcinogenic
4. bacteristatic
5. resistance- 50s subunit doesn't bind drug
6. spectrum- mostly affects G+

c. lincomycin

1. inhibits peptide bond formation

d. clindamycin

1. inhibits peptide bond formation
2. used for anaerobic infections (which are hard to treat)

F. those that work by competitive inhibition

1. inhibit one enzyme of pathway with substrate analogs
2. they inhibit tetrahydrofolate (THFA) synthesis
 - a. one step in the process from GTP to THFA involves taking in PABA
 1. PABA=para amino benzoic acid
 - b. synthetase is the enzyme that links PABA to the previous substrate
 - c. later on there's a reduction reaction catalyzed by reductase
 - d. can make PABA analogs
 1. sulfa drugs are PABA analogs
 - A. they inhibit synthetase—> no THFA—> no methionine or thymine
 1. this stops bac growth
 - B. ex:
 1. para amino benzene sulfonamide
 2. para amino salicylic acid
 - a. it's an antitubercular sulfa drug
 2. bactrim = sulfamethoxazole (a sulfa drug) + trimethoprim
 - A. sulfamethoxazole inhibits synthetase while trimethoprim inhibits reductase.

G. summary of antitubercular Drugs

1. D-cycloserine
2. rifampicin
3. streptomycin
4. para amino salicylic acid

IX. Sensitivity Testing

A. when patient has infection, don't try 1 drug after a/t; instead, test resistancy to antibiotics b/f

B. use Kirby-Bauer Technique

1. involves agar diffusion- drug diffusing out on plate
2. technique is highly standardized w/ regard to org, drug, inoculant size (hoe many cells are put down initially, incubation time)
3. get sample from patient's infection site, isolate bac, and swab plate (lawn procedure)
4. use Mueller-Hinton agar (does not contain a/t that will work against drugs' reactions)
5. add multiple discs w/ dif antibiotics to dif sections of plate
6. measure diameters of zones of inhibition for each drug in millimeters (w/ machine)
7. record results as:
 - a. sensitive- org responds to usual dose (enough to kill org, not enough to be toxic, taking into account 4 factors that could affect availability)
 - b. intermediate- requires higher dose than usual
 - c. resistant- can't kill bac at achievable blood levels
 1. org is not resistant w/ usual drug mechanisms
 2. drug can get in and kill bac in test tube, but b/c of 4 factors preventing drug from reaching bloodstream, can't get enough dose into bloodstream to cure infection
8. there's a chart listing size of zones of inhibition and says if drug is sensitive, intermediate, and resistant- can't decide w/o looking at chart

X. Other Microorganisms

A. fungi

1. eukaryotes
 - a. have nuclear membranes
 - b. undergo mitosis
 - c. have membranous organelles
 1. ER
 2. Golgi apparatus
 3. mitochondria
 - d. do have cell walls though many eukaryotes don't
 - e. can exist in multicellular forms
2. classification
 - a. macroscopic fungi (mushrooms)
 1. most are non-pathogenic, though some are
 - b. microscopic fungi (yeast, molds)
 1. yeasts
 - A. single-celled organisms
 1. can grow in petri dish, streak for single colonies, count them- like bac
 - B. divide by budding
 1. forms a bud which breaks off, similar to bac, but does have mitosis
 2. molds
 - A. characterized by presence of hyphae
 1. long tubular filaments
 2. multiple nuclei in cytoplasm
 3. 2 types:
 - a. septate- have septa
 - b. nonsepatae- no septa
 - B. grow in network = mycelium (fuzzy green stuff on moldy fruit or bread)
 1. branched hyphae grow until become visible and large

3. nutrition

- a. they are heterotrophic- grow on organic molecules as C-sources
(as opposed to autotrophic org that are photosynthetic and get C from inorganic carbon dioxide)
 - 1. 2 types:
 - A. saprophytic- get nutrients from dead animals or plants
 - B. parasitic- get nutrients from living organisms

4. reproduction

- a. asexual
 - 1. partitioning of hyphae (for mold not yeasts)
- b. sexual
 - 1. male and female forms of fungal cell fuse together and form spores which =
 - A. survival- can survive adverse conditions
 - B. spread- can spread easily and form new colonies (can become health problem)
 - 1. molds can get into lungs and cause respiratory diseases
 - 2. they also produce toxins that get into air and cause medical problems

B. parasites

- 1. generally considered to be animal cells
 - a. ex = amoeba
 - b. no cell wall
 - c. motile
- 2. heterotrophic
- 3. free-living- in soil, water...
- 4. reproduction
 - a. asexual- similar to binary fission
 - b. sexual- male and female forms, fusion—>zygotes
- 5. a large number of parasites are single-celled, but there are multicellular parasites:
 - a. flatworms
 - 1. ex: tapeworms- thin, can be 25-30 ft long
 - b. roundworms
 - 1. ex: trichina- a few inches long, live in muscles, associated w/ pork

C. viruses (animal viruses not bac viruses)

- 1. smaller than cells
- 2. they're not cells
 - a. no cytoplasm
 - b. no organelles (no rib, mitochondria...)
 - c. no locomotion/motility
 - d. no metabolism
- 3. they're nucleic acids (DNA, RNA) within a protein coat
 - a. some have a lipid envelope surrounding the protein coat (resembles a membrane)
- 4. they are parasites, specifically obligate intracellular parasites
 - a. parasites- general bio term which means dependent upon some other org for its existence
 - b. ultimate parasite b/c can't exist except in living cells (for metabolism, prot synthesis, disease)
 - c. there are viruses that infect plants, animals, protozoa, and fungi
- 5. replication
 - a. attachment (specificity- rec on surface of cell match prot on surface of virus)
 - b. penetration (s/t whole viral particle goes in, s/t just nucleic acid- like w/ phage)
 - c. virus controls cell's genetic and metabolic function
 - 1. makes new viral DNA, viral proteins...
 - 2. assembly
 - 3. release (s/t lysis, s/t exocytosis w/o destroying cell)
 - 4. s/t go through lysogenic state instead

XI. Brief Survey of Infectious Org and the Diseases They Cause

A. bacteria

1. group A streptococcus- causes strep throat
2. staphylococcus- causes skin lesions (boils, blisters) and food poisoning
3. clostridium- causes tetanus and botulism
4. streptococcus pneumoniae- causes pneumonia (fluid in lungs)
5. salmonella- causes food poisoning
6. vibrio cholerae- causes cholera→massive diarrhea→dehydration- loss of fluid and Na and K ions
 - a. treatment- rehydration and electrolytes
7. bacillus anthracis- causes anthrax

B. fungi

1. cutaneous- skin
 - a. ex: dermatophytes- causes skin, hair, and nail diseases (ex: athlete's foot)
2. subcutaneous- under surface of skin
3. systemic- worst type

C. parasites

1. entamebahistoltytica- causes amebiasis (a form of dysentery = diarrhea disease)
 - a. 500 million ppl suffering from it in tropics
2. plasmodium- causes malaria (fever, chills...)
 - a. 500 million ppl suffering from it

D. viruses

1. DNA viruses
 - a. poxvirus- causes smallpox
 - b. papilloma virus- causes warts
2. RNA viruses
 - a. poliovirus- causes polio
 - b. rhinovirus- causes common colds
 - c. influenza- causes the flu
 - d. mumps, measles, rabies
 - e. AIDs
3. DNA and RNA tumor viruses can cause cancer

XII. Pathogenic Mechanisms

A. reservoir- what is the natural habitat of these organisms?

1. air
2. water
3. soil
4. food
5. surface of skin
6. ppl
7. animals

B. transmission- how do bac get from reservoir to inside ppl?

1. breathe air
2. soil on hand
3. drink water
4. eat food
5. skin contact- STDs

C. portal of entry- from where do they get into body?

1. anywhere where epithelial tissue meets the surface
 - a. upper respiratory tract (open mouth and can get strep throat...)
 - b. lower respiratory tract (breathe in further and can get pneumonia, tuberculosis...)

- c. gastrointestinal tract (mouth—>stomach—>intestines)
- d. excretory system (epith tissue—>outside world, bac can get in from urethra)
- e. urogenital system (urinary tract infections, venereal diseases!; catheters can lead to disease)
- f. break in skin

- 1. bruise
- 2. contaminated needle
- 3. insect bite (insects transfer bac from one source to another)

D. portal of exit

- 1. sneeze, cough, spit- eject org from respiratory tract
- 2. feces (talking about org from intestinal tract)
- 3. urine (urinary tract infections)
- 4. blood (blood infections)

E. factors- dose

- 1. single org will not get one sick- need minimal number (dose) to cause clinical disease
- 2. can have org and not be sick- can even affect other ppl b/f one knows he's sick

F. process

- 1. adhesion (common pili)- bac have to adhere to cause disease
- 2. uptake- human has to take up bac cell
- 3. causes disease due to virulence factors:
 - a. toxins
 - 1. endotoxins- ex: lipid A
 - 2. exotoxins
 - A. enterotoxins- cause intestinal disease
 - B. neurotoxins- affect nervous system (spastic paralysis, flaccid paralysis...)
 - b. enzymes
 - 1. digestion of host tissues
 - 2. break up clots, enabling the org to spread
 - 3. make a clot so org does not get diluted
 - 4. hemolysins destroy RBC (could be listed under toxins, too)
 - c. avoid host defenses
 - 1. hide
 - A. in biofilms
 - B. intracellular growth- hide in human cells
 - 1. protected from antibodies, drugs, & phagocytosis
 - 2. resist phagocytosis using capsules
 - 3. kill phagocytes by producing leukocidins (which kill WBC)

G. stages of disease

- 1. incubation period- time of contact until show symptoms
- 2. prodromium- initial, vague symptoms
- 3. invasion (acute)- bugs multiple, adequate dose to cause clinical symptoms, severest symptoms
- 4. convalescent period- period of recovery, symptoms recede

H. terms of infection

- 1. local vs. systemic
 - a. local- at site where bac first came in
 - b. systemic- throughout whole body
- 2. primary vs. secondary
 - a. primary- what patient came in w/ to begin w/
 - b. secondary- new infection that arises after primary inf(ex: get pneumonia from respirator)
- 3. acute vs. chronic
 - a. acute- sudden onset, very severe, short time frame
 - b. chronic- long time frame

I. effects of infection

1. signs- measurable properties (ex: WBC count, skin lesions, temp, BP)
2. symptoms- subjective properties (ex: pain, fatigue, nausea)
3. syndrome- predictive complex of signs and symptoms

XIII. Microbiology Can be Used for Good

A. food and drinks

1. bread

a. 3 purposes of microorganisms:

1. to leaven dough- make it rise

- A. add baker's yeast = *Saccharomyces cerevisiae*
- B. flour contains starch which is broken down into sugar by yeast's exoenzymes
- C. sugar enters yeast cell and goes through glycolysis and Krebs'- aerobic respir.
- D. this produces carbon dioxide which helps the dough rise
- E. activity depends upon 4 factors:
 1. inoculum (how many yeast cells)
 2. sugar conc
 3. temp
 4. salt conc

2. to condition dough- make it soft and flexible so can knead it

- A. yeast exoenzymes break flour proteins (especially gluten) to make the dough soft = elastic dough
- B. then can knead the dough which brings in more air b/c more surface area, so can undergo aerobic respiration

3. to give the dough flavor and odor

- A. ex: in rye bread, add lactic acid bac
- B. they produce acids and alcohols that give flavor to bread- make it pungent

2. cheese

a. it's the lactic fermentation of milk

b. add starter culture of bac to milk which adds flavor and produces acid.

1. the acid coagulates milk proteins which gives clumps called curd

c. also add renin- an enzyme from a calf's stomach

1. helps coagulate proteins

d. curd is:

1. heated and pressed which removes the water/whey
2. salted
3. ripened for 1-16 months

e. different types of cheese:

1. soft

- A. cottage- use *Lactococcus lactis*
- B. cream- use *Lactococcus cremoris*

2. semisoft

- A. Limberger- use *Lactococcus lactis*
- B. muenster- use *Lactococcus lactis*

3. hard

- A. cheddar- use *Lactococcus lactis*
- B. edam- use *Lactococcus lactis*
- C. gouda- use *Lactococcus lactis*
- D. swiss- use *Lactococcus lactis* and propioni bacteria

1. prop bac prod carbon dioxide through respiration which produces holes

4. very hard

- A. parmesan- use *Lactococcus lactis* and *Lactococcus bulgaricus*

3. fermented milk

a. procedure:

1. inoculate with desired culture
2. incubate at optimum temp
3. stop growth by cooling

b. use *Lactobacillus* and *Lactococcus lactis*

1. they produce an acid and aroma

c. also use *Lactococcus lactis* subspecies *diacetylactis*

1. this converts citrate into diacetyl which gives it a buttery flavor

d. products of this procedure:

1. skim milk—>butter milk
2. cream—>sour cream

4. yogurt

a. use *Streptococcus thermophilus*

1. this produces an acid that turns liquid milk into thick yogurt

b. also use *Lactobacillus bulgaricus*

1. this produces the aroma

c. fresh yogurt has 1 billion bac/gram

5. wine

a. procedure:

1. crush grapes
2. then have a choice:
 - A. can separate liquid/must which prod a white juice used to make white wine
 - B. leave in skins which prod a juice used to make red wine
3. inoculate the grape juice one of 2 ways:
 - A. can use yeast org on skins of grapes
 1. prob- it's unpredictable. S/t works. S/t doesn't. Gives dif flavors.
 - B. can use sulfur dioxide to kill yeast org & add *saccharomyces cerevisiae* (yeast)
4. allow fermentation to take place in anaerobic conditions for 3-5 days
 - A. this causes yeast to produce alcohol
5. age the wine- let it sit for a while which clears the wine
 - A. this gets rid of the sediment and allows for the development of flavor

b. products:

1. dry wine
 - A. no sugar b/c yeast eats it up
2. sweet wine
 - A. high sugar conc originally
 - B. alcohol inhibits growth of yeast cells prod it b/f the sugar is used up
3. wine vinegar/cooking wine
 - A. add *Acetobacter* which produces acetic acid/vinegar
4. champagne and sparkling wine
 - A. continue fermentation in the bottle which causes yeast to prod carbon dioxide

B. industrial fermentation

1. can grow a huge tank of bac to get their products

2. bac, yeast, and molds are clever organic chemists

a. primary metabolites- normal products of metabolism

1. amino acids, ethanol, citric acid

b. secondary metabolites- under certain conditions, bac can make other products:

1. bac and mold can make antibiotics
2. can make biopolymers- long chains of molecules, such as polysaccharides
 - A. can be used to give texture of ice cream

XIV. Bacteria Can be Used for Evil

A. bioterrorism

1. the intentional use of microorganisms and toxins of microbial, plant, or animal origin to produce disease and/or death in humans, livestock, or crops

B. Why use bioweapons?

1. low production cost
 - a. nuclear and chem prod are expensive, while bioweapons can be made in a basement lab
2. nondetection by routine security systems
 - a. know when bombs are coming, but bac are invisible and weightless- hard to detect
3. easy transportation
4. easy access to many agents
 - a. can get them in the field- ex: anthrax from soil around dead cows
 - b. can also get them from American Type Culture Collection (ATCC)- has every type of org
5. destroy ppl, not buildings
 - a. so bad guys can take over after they kill the opposing army
6. can kill more (compared to nuclear and chemical weapons)
 - a. whoever is originally exposed will expose most of the population
7. easy delivery
 - a. can put bac in an aerosol can and spray them in subway
 - b. crop dusters can spray bioweapons instead of fertilizer
8. much is published and known
 - a. bad guys read journals, too, and can use genetic engineering to make bac resistant to antibiotics and vaccines
 - b. this brings up discussion of censorship
 1. no external censorship (no rules)
 2. yes internal censorship- ppl realize on their own not to publish their findings

C. bioweapons is not a new idea

1. armies would dip arrows into feces, animal poisons, etc.
2. armies would catapult dead bodies of ppl who died from smallpox or bubonic plague- spread disease
3. Am and Eng infected blankets w/ smallpox and measles and traded w/ Indians to spread disease
4. Fort Detrick- in suburbs of Washington is a germ warfare labarotory
 - a. developed most potent bioweapons during Cold War for purposes of attacking enemy
5. Novosibersk- Soviet Union's germ warfare lab during the Cold War
6. 1972- Biological and Toxins Weapons Convention (treaty)
 - a. all countries got together to ban germ warfare
7. 1988- Soviet Union had 100 tons of anthrax
 - a. 2 g can kill 10,000 ppl
 - b. anthrax was put in steel containers w/ bleach and buried it on an island 700 miles off coast
 - c. containers started to rust, and island moved closer to the mainland- animals go back and forth
8. Iraq had weapons of mass destruction- 5,000 galllons of botulinum, 2000 gal of anthrax
9. Iran, Syria, N. Korea, and China also have germ warfare labs
10. Al Qaeida- terrorist org can get ahold of them

D. agents- bac, viruses, toxins

1. bacteria

disease	org that causes it	infectious dose	incubation period	illness duration	lethality	persistance
inhalation anthrax	Bacillus anthracis	8,000-50,000 spores	1-6 days	3-5 days usually fatal	high	40 yrs

brucellosis	Brucellasis	10-100 org	1-2 months	weeks	low	stable
pneumonic plague	Yersinia pestis	100-500 org	2-3 days	1-6 days usually fatal	high	1 yr
tularemia	Francisella tularensis	10-50 org	3-5 days	2 weeks	_	months

2. viruses

smallpox	variola virus	high transmission at low dose	7-17 days	4 weeks	high	_
----------	---------------	-------------------------------	-----------	---------	------	---

3. toxins

botulism	Clostridium botulinum	.001 micrograms/kg	1-5 days	death in 24 hrs	high	weeks
ricin	castor bean	3-5 micrograms/kg	18-24 hrs	10-12 days usually fatal	high	stable

E. targets

1. ppl
2. livestock- cattle, sheep
3. crops- has many implications:
 - a. economics- farmers depends on it; exports are needed
 - b. food supply- all stores tog only have enough food for 5 days, so dependent on food coming into city
 - c. ethical issue: food insecurity- not enough food - should one share it or keep it for himself?

F. solutions

1. political and military- can try to prevent it from happening
2. if attacked...
 - a. detection- don't wait until ppl die to notice attack
 1. there are high-tech detection methods- sensors and detectors pick up presence of org
 - b. communication- comp network throughout US, so can warn e/o and share info
 - c. diagnostic labs- hospitals, public health facilities, CDC (Center for Disease Control), USAMRIID (US Army Medical Research Institute of Infectious Diseases)- most effective
 1. USAMRIID was Fort Detrick- changed name and now do defense instead of offens
3. treatment
 - a. antibiotics- but there are prob w/ them:
 1. doses- how do you make so many?
 2. distribution- where do you store antibiotics and who should get them?
 3. don't have antibiotics b/c these are oddball org
 - A. so govt has to pay ppl to make them- Project Bioshield
 - b. vaccines- same prob as antibiotics- kinds, numbers, distribution
4. w/ regard to public health
 - a. need to train 1st responders to recognize the disease when see it and to know how to treat it
 - b. attacks may overwhelm the system b/c don't have enough beds in the hospital
 1. may need to convert football fields into makeshift hospitals
5. on the personal level
 - a. if attacked, stay indoors, close windows, seal AC unit, seal room w/ duct tape, wear gas masks, pray