Transcript CIC Course

Identification of Infectious Disease Process

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Slide 1: Good morning. My name is Jessie Seidelman, and I am one of the ID faculty from Duke. I am also one of the medical directors for Duke University hospital. I am very excited to speak with you today regarding the identification of the infectious disease process.

Slide 2: I will disclose that I receive royalties from UpToDate. I also want to acknowledge Dr. Rebekah Moehring for these slides.

Slide 3: Here are the objectives of this talk. We will be covering pathogenesis, immune process, diagnostics and laboratory reports, true infection versus colonization or contamination and lastly antimicrobial use.

Slide 4: I have a few more disclosures for all of you. First, this is only a 90- minute session. People including myself spend years learning about infectious diseases and microbiology. As such, I am going to address each core principle broadly and add some context. I will do my best to hit key pathogens from all the 38 other chapters in the study guide, but again, there is so much more to all of these topics than we can cover in a short session.

Slide 5: Ok, let's start with the infectious disease process or pathogenesis.

Slide 6: So, what leads to a person getting an infection? This is really an interaction between three key factors: the host, the pathogen, and the environment, which is affectionately referred to as the transmission triangle.

Slide 7: Specifically, the factors that contribute to infection acquisition are the host's immune system. Are they susceptible to infection? How virulent a pathogen is in terms of invasion and ability to evade the immune system. Lastly is the environmental exposure. Is the exposure significant enough to allow pathogen entry into the host and are there enough infection units acquired to cause an infection?

Slide 8: Let's specifically dive into the environmental risk factors. This specifically includes the people, places and time spent in a specific area. Another way to stratify the environment as it related to infectious diseases is healthcare versus community settings.

Each setting has its own unique environmental risk to consider for example the likelihood of a contamination event. The likelihood of an encounter with a returning traveler with a potentially transmissible infection or even what time of the year should be considered when thinking about environmental risk factors.

Slide 9: So, let's think about how transmission may occur via the environment.

E coli is part of patient 1's gut microbiome. Patient 1 had a BM that leads to E coli contamination of the bed. The nurse for patient 1 touches the bed but does not effectively clean his or her hands. Patient 2 is elderly with impaired immunity and poor functional status. The nurse then does peri care on patient 2's foley catheter. E coli contaminates patient 2's catheter and patient 2 then develops a catheter associated urinary tract infection.

Slide 10: However, the transmission process may not always be this linear. There may be multiple intermediate steps including reservoirs and intermediaries. The best examples of this phenomena are illustrated by the CDC life cycle diagrams. Here is one example of malaria where you can see the mosquito reservoir, how the parasite reproduces in the mosquito and exits the reservoir into the human. This is important in thinking about potential exposures and incubation periods.

The reservoir of an infectious agent is the habitat in which the agent normally lives, grows, and multiplies. Reservoirs include humans, animals, and the environment. The reservoir may or may not be the source from which an agent is transferred to a host. For example, the reservoir of *Clostridium botulinum* is soil, but the source of most botulism infections is improperly canned food containing *C. botulinum* spores.

Many common infectious diseases have human reservoirs. Diseases that are transmitted from person to person without intermediaries include sexually transmitted diseases, measles, mumps, streptococcal infection, and many respiratory pathogens. Because humans were the only reservoir for the smallpox virus, naturally occurring smallpox was eradicated after the last human case was identified and isolated.

Humans are also subject to diseases that have animal reservoirs. Many of these diseases are transmitted from animal to animal, with humans as incidental hosts.

Plants, soil, and water in the environment are also reservoirs for some infectious agents. Many fungal agents, such as those that cause histoplasmosis, live and multiply in the soil. Outbreaks of Legionnaires disease are often traced to water supplies in cooling towers and evaporative condensers, reservoirs for the causative organism *Legionella pneumophila.*

The portal of exit is the path by which a pathogen leaves its host. The portal of exit usually corresponds to the site where the pathogen is localized. For example, influenza viruses and *Mycobacterium tuberculosis* exit the respiratory tract, schistosomes through urine, cholera vibrio in feces, *Sarcoptes scabiei* in scabies skin lesions, and enterovirus 70, a cause of hemorrhagic conjunctivitis, in conjunctival secretions. Some bloodborne agents can exit by crossing the placenta from mother to fetus (rubella, syphilis, toxoplasmosis), while others exit through cuts or needles in the skin (hepatitis B) or blood-sucking arthropods (malaria).

The portal of entry refers to the way a pathogen enters a susceptible host. The portal of entry must provide access to tissues in which the pathogen can multiply, or a toxin can act. Often, infectious agents use the same portal to enter a new host that they used to exit the source host. For example, influenza virus exits the respiratory tract of the source host and enters the respiratory tract of the new host. In contrast, many pathogens that cause gastroenteritis follow a so-called “fecal-oral” route because they exit the source host in feces, are carried on inadequately washed hands to a vehicle such as food, water, or utensil, and enter a new host through the mouth.

Slide 11: There are a few important definitions and descriptions to consider when it comes to talking about transmissibility of pathogens.

First, inoculum is the number of organisms needed to cause a disease during exposure. Norovirus is very infectious, only 10-100 viral particles may be sufficient to infect an individual.

The other thing to consider is how well the pathogen survives in the environment. Again, some pathogens can remain viable in the environment for weeks to months. For instance, C difficile spores are resistant to alcohol and can remain viable in the environment for months.

Then there is the method of the transmission. Infectious agents may be transmitted from its natural reservoir to a susceptible host in different ways. There are different classifications for modes of transmission. Direct versus indirect. In direct transmission, an infectious agent is transferred from a reservoir to a susceptible host by direct contact or droplet spread. **Direct contact** occurs through skin-to-skin contact, kissing, and sexual intercourse. Direct contact also refers to contact with soil or vegetation harboring infectious organisms. Thus, infectious mononucleosis ("kissing disease") and gonorrhea are spread from person to person by direct contact. Hookworm is spread by direct contact with contaminated soil. **Droplet spread** refers to spray with relatively large, short-range aerosols produced by sneezing, coughing, or even talking. Droplet spread is classified as direct because transmission is by direct spray over a few feet before the droplets fall to the ground. Pertussis and meningococcal infection are examples of diseases transmitted from an infectious patient to a susceptible host by droplet spread. **Indirect transmission** refers to the transfer of an infectious agent from a reservoir to a host by suspended air particles, inanimate objects (vehicles), or animate intermediaries (vectors).

Lastly is the reproductive number. This is the expected number of cases directly generated by one case in a population where all individuals are susceptible to infection. The definition assumes that no other individuals are infected or immunized.

Slide 12: Practice Question- *Answer B. Adults shed flu virus from the DAY BEFORE symptoms begin through 5-10 days after illness onset. Incubation for flu is 1 to 4 days (average 2 days).*

Slide 13: Here is the diagram that really puts a lot of the concepts we just talked about together

- Think about the susceptibility of the host does the host lack the immunity or physical resistant to overcome invasion from the pathogen.
- Next, think about the actual pathogen. As we talked about previously this includes the virulence or infectiousness, the ability of the organism to survive in the environment, and how many bacteria, virions, parasites etc. are able to cause an infection.
- The next part is the reservoir. Where does the pathogen reproduce and thrive?
- What is the portal of exit from the reservoir?
- Next is the transmission from the portal of exit to the host and finally the portal of entry into the host.

Slide 14: So, what can we do to mitigate transmission of pathogens to hosts? This includes environmental engineering, cleaning and disinfection, avoidance of presenteeism, in other words staying home when you are sick. Appropriate use of transmission-based precautions, hand hygiene and patient cohorting and appropriate staffing ratios.

Unfortunately, there are also factors that we cannot modify within infection prevention. That includes the hosts, geographic or regional epidemiology, and things like chemotherapy, invasive devices, or surgery.

Slide 15: Practice Question-*Answer B. As we talked about portal of entry.*

Slide 16: Ok so now we are moving onto the host.

Nonspecific factors that defend against infection include the skin, mucous membranes, gastric acidity, cilia in the respiratory tract, the cough reflex, and nonspecific immune response. Factors that may increase susceptibility to infection by disrupting host defenses include malnutrition, alcoholism, and disease or therapy that impairs the nonspecific immune response.

When it comes to the immune system specifically there are three different components. An individual's genetic makeup may either increase or decrease susceptibility. For example, people with sickle cell traits seem to be at least partially protected from a particular type of malaria. Specific immunity refers to protective antibodies that are directed against a specific agent. Such antibodies may develop in response to infection, vaccine, or toxoid (toxin that has been deactivated but retains its capacity to stimulate production of toxin antibodies) or may be acquired by transplacental transfer from mother to fetus or by injection of antitoxin or immune globulin.

Slide 17: Practice Question- *Answer C. Neutrophil*

Monocytes. They have a longer lifespan than many white blood cells and help to break down bacteria.

Lymphocytes. They create antibodies to fight against bacteria, viruses, and other potentially harmful invaders.

Neutrophils. They kill and digest bacteria and fungi. They are the most numerous types of white blood cell and your first line of defense when infection strikes.

Eosinophils. They attack and kill parasites and cancer cells and help with allergic responses.

As a bonus WBC **Basophils.** These small cells seem to sound an alarm when infectious agents invade your blood. They secrete chemicals, such as histamine, a marker of allergic disease, that help control the body's immune response.

Slide 18: In terms of immunity here are some terms and numbers to know

A normal WBC range is from 4,000 to 10,000 cells per mm cubed. However, this is more often displayed as 4.0 to 10.0. When the WBC is higher than 10 it is referred to as leukocytosis. When the WBC is lower than 4 it is referred to as leukopenia. Neutropenia is referring to low neutrophils. The specific definition is when the absolute neutrophils count is less than 1000 or when polys plus bands are less than 500. You can see below some additional definitions of the absolute neutrophil count, the polys or PMNs and the bands.

Infection risk is high when the absolute neutrophil count is less than 500.

Slide 19: Two types of immunity exist — active and passive:

Active immunity occurs when our own immune system is responsible for protecting us from a pathogen. Active immunity is created by our own immune system when we are exposed to a potential disease-causing agent (i.e., pathogen). Active immunity is important because it lasts a long time in the form of immunologic memory. Immunologic memory consists of B and T cells that can recognize a particular pathogen. Vaccines contribute to active immunity by providing us with a controlled way to create an immune response. When a vaccine is introduced, our immune system treats it like any other exposure. It works to stop the "assault", and, in the process, immunologic memory develops. Because vaccines are

designed such that they do not cause illness, we gain the benefits of the exposure without the risks associated with fighting off a natural infection.

Passive immunity occurs when we are protected from a pathogen by immunity gained from someone else. Passive immunity, or immunity gained in a way other than from one's own immune system, can occur in a few ways and can be lifesaving. However, passive immunity is short-lived because the antibodies are not continually replenished as they would be in an individual whose immune system is responding directly. Passive immunity can occur in a couple of ways: maternal antibiotics, IVIG.

A third category, community immunity, does not involve physical components of the immune system for protection, but is still worth discussion in this capacity. Community immunity occurs when people are protected by those around them. This type of protection is indirect in that it does not involve physical components of immunity, such as antibodies, but rather results when a pathogen is less likely to infect a susceptible person because of the high numbers of protected people around them. Because this immunity is not based on "products" of the immune system, it is the least reliable. However, for some in our communities, such as those too young to be immunized or those with weakened immunity due to illness or treatment, community immunity is the only way they can be protected. This includes the notion of herd immunity.

Slide 20: There are five main classes of immunoglobulins—IgG, IgM, IgA, IgD, and IgE

IgM is predominantly found in lymph fluid and blood and is a very effective neutralizing agent in the early stages of disease. Elevated levels can be a sign of recent infection or exposure to antigen.

IgG is the major immunoglobulin in blood, lymph fluid, cerebrospinal fluid and peritoneal fluid and a key player in the humoral immune response. IgG is produced in a delayed response to an infection and can be retained in the body for a long time. The longevity in serum makes IgG most useful for passive immunization by transfer of this antibody. Detection of IgG usually indicates a prior infection or vaccination. IgG has 4 subclasses. Determination of IgG subclasses can be a valuable tool in indicating a potential antibody deficiency. Selective IgG subclass deficiencies are associated with disease.

Immunoglobulin A (IgA) is the most abundant type of antibody in the body, comprising most of the immunoglobulin in secretions and a significant amount of circulating immunoglobulin. In secretions, it serves to protect the mucosal tissues from microbial invasion and maintain immune homeostasis with the microbiota.

IgE and IgD are found in serum in much smaller quantities than other Ig classes. IgE primarily defends against parasitic invasion and is responsible for allergic reactions. Membrane IgD is a receptor for antigens found mostly on mature B-lymphocytes.

Slide 21: Practice Question- *Answer B. I always think M is for Immediate*

Slide 22: Here's a slide to help show you when IgG and IgM develop in acute hepatitis A infection. You can see that the IgM peaks and then declines and the IgG slowly increases over time. Knowing the timing of when the body produces these immunoglobulins can be helpful to know if a patient is acutely or chronically infected.

Slide 23: Here is an example of a table that is one that you should save somewhere for reference. It is helpful in diagnosing if a patient has acute hepatitis, A, acute hepatitis B, acute hepatitis C, or coinfections.

I'll start by going through the serologies at the top. Hepatitis B surface antigen or HBsAg is a protein on the surface of hepatitis B virus that can be detected in high levels in serum during acute or chronic hepatitis B virus infection. The presence of HBsAg indicates that the person is infectious, except when it might be transiently positive within 30 days after a dose of hepatitis B vaccine (HepB). The body normally produces antibodies to HBsAg as part of the normal immune response to infection.

IgM Anti-HAV is going to indicate an acute hepatitis A infection.

IgM Anti-HBC Positivity indicates recent infection with hepatitis B virus (<6 mos). Its presence indicates acute infection. IgM anti-HBc should be ordered only when acute HBV infection is a concern.

Anti HCV is going to be antibodies against hepatitis C.

Slide 24: Practice Question- *Answer B. Hepatitis D virus (HDV) infection is caused by a defective virus: the hepatitis D virus. HDV is often referred to as hepatitis delta virus or delta agent. However, the term HDV is preferred. Individuals with hepatitis D are always dually infected with HDV and hepatitis B virus (HBV). Although HDV can replicate autonomously, the simultaneous presence of HBV is required for complete virion assembly and secretion.*

Slide 25: Practice Question – *Answer D. We hope these guys have developed their donor's immunity at 1 year following bone marrow transplant. All the other scenarios describe patients that are immunocompromised.*

Slide 26: Ok so what is a susceptible host? This can really include a large variety of clinical elements. It can be something as simple as the patient hasn't been exposed to a specific virus, so they don't have any adaptive immunity. They could have had an invasive procedure. By disrupting the gut or skin barriers that can make you more susceptible to bacteremia translocating to the wrong place. There are also many medications and diseases that put patients at increased risk of infection. Specifically, this could be medications like high dose steroids or chemotherapy. This could also be leukemias that reduces your number of functional neutrophils. Even metabolic disorders such as diabetes can increase your risk of infection. HIV or AIDS puts you at risk of opportunistic infections. Similarly, if you have asplenia or functional asplenia from sick cell disease then your body cannot control the production and removal of blood elements. Lastly, inherited immune deficiency disorders such as Severe Combined Immunodeficiency (SCID) can put an individual at risk for infections.

Slide 27: Ok so we have talked about the environment. We have talked about the host. Now it is time to bring it home and talk about the pathogen.

So, there are different factors that contribute to its ability to invade the host, evade host immunity or survive.

Certain bacteria may produce enzymes to increase local tissue damage to promote their spread such as strep pyogenes or group A strep toxin in the setting of necrotizing fasciitis. Some bacteria are very motile and, in that way, can invade and easily disseminate such as E coli's ability to swim up a ureter. Biofilms are a great way that bacteria can evade host defenses. This happens a lot with indwelling prosthetic devices such as coagulase negative staph on an IV line or staph aureus in a prosthetic knee infection. Some bacteria can also live in very harsh conditions. For example, in C difficle or bacillus species they can live in a spore form which is hardy and can survive for weeks to months out in the environment.

Slide 28: In terms of specific pathogen features that you need to know here is a brief list. You have to know whether the microorganism is a bacteria, virus, fungus, parasite or a prion. How do these pathogens clinically manifest themselves? What are the correct studies that we should send to the lab to evaluate for them? What are the correct precautions that a patient should have in a healthcare setting if they are being treated for or suspected of having one of these pathogens? The timing and type of precautions really depends on how the microorganism is transmitted and when it is transmitted.

Slide 29: Here is a general list that you can reference when you are studying for your exam. Again, you want to know what is causing the illness, how it presents, what is used to test for it, and what precautions a patient might need and for how long.

Slide 30: Here are some quick microbiology correlations that may help you think about where the infection is coming from.

When it comes to gram positive organisms, think about the skin, the lungs, the GI tract, and implanted devices. When it comes to gram negatives, think about the GI tract, urine, and some lung pathogens. Atypical pathogens that don't have true cell walls like legionella, mycoplasma, and chlamydia. Think about the lung, sexually transmitted infections and tick born illnesses. When it comes to anaerobic pathogens, think of gas and abscess forming clinical pictures, bad odor wounds and the GI tract. Less commonly encountered pathogens may be mycobacteria and spirochetes such as borrelia. I am not sure that syphilis in generally is less commonly encountered but perhaps in the inpatient IP setting it may be. Lastly, fungal pathogens think of guts, devices, particularly if a patient is receiving total parenteral nutrition and really immunocompromised individuals.

Slide 31: So how does the identification of organisms actually happen in the lab? Often times a gram stain is performed and this can give you information pretty quickly. The gram stain can tell you if the organism is gram positive (will be purple), gram negative (will be pink) or non-staining. You will also be able to see the shape of these pathogens that will give you good clues as to what you are dealing with. Is it a coccus or a circle, a rod, a spiral or a square.

Slide 32: The gram stain is a very old technology but incredibly important to you as IPs and also to clinicians who are trying to empirically treat patients. Knowing the shape and gram stain can help guide early empiric antibiotic choices.

Slide 33: Practice Question – *Answer D. Remember staph is from the Greek word staphyle (bunch of grapes) and kokkos (berry),*

Slide 34: There are other things about the bacteria that you need to know if you are going to try and grow them up in the lab and isolate them. Specifically, there are specific physical requirements for growth of bacteria. They need specific media to grow that contain various nutritional elements. There also needs to be controlled temperature and atmospheric conditions. Aerobic bacteria need oxygen versus anaerobic bacteria that need there to be NO ~~bacteria~~ oxygen in order to survive. Facultative anaerobes can grow in either. Microaerophilic means they require lower levels of oxygen than are present in the atmosphere to survive.

Slide 35: So here is another good slide for reference. Here you have what growth media with support which pathogens. I will say that the standard agar is blood agar. MacConkey is for gram negative pathogens. The rest of them are selective agar meaning that they are only going to support specific pathogens for

growth. The other thing I have on this slide is the biochemical tests. For the catalase test they basically add hydrogen peroxide to the plate and see if it produces bubbles. However, it should never be performed on organisms that have been grown on blood agar (a medium that contains blood). This is because there is a catalase activity in blood that would produce a false positive result. The other test is the coagulase test. The coagulase test is one way to differentiate the highly pathogenic S. aureus from the other less pathogenic staphylococcal species. If positive you will basically see cells clumping together on the slide.

Slide 36: Some other key microbiology grouping is whether the bacteria cause hemolysis or not. The key difference between alpha beta and gamma hemolysis is that alpha hemolysis is the partial destruction of red blood cells in the blood and beta hemolysis is the complete destruction of red blood cells in the blood, while gamma hemolysis does not involve any breakdown of red blood cells. This equates to the visualization of the bacteria on the plates as clear (complete hemolysis), green (partial hemolysis) and red (no hemolysis)

Other keywords are the lancefield grouping. This is a system of classification that classifies catalase-negative Gram-positive cocci based on the carbohydrate composition of bacterial antigens found on their cell walls.

The bile-esculin test is widely used to differentiate enterococci and group D streptococci, which are bile tolerant and can hydrolyze esculin to esculetin, from non-group D viridians group streptococci, which grow poorly on bile.

Optochin is a chemical that is toxic to some bacteria but harmless to others. It is useful in the identification of ***Streptococcus pneumoniae***, the alpha-hemolytic Streptococcus most commonly susceptible to this chemical.

Slide 37: Let's talk a little bit about common gram-positive organisms. Again, these are purple on the stain. When it comes to gram positive cocci these include staph aureus (clusters), coagulase negative staphylococcus, streptococcus pneumoniae, streptococcus, and enterococcus. Again, you can see why we may need some of those biochemical tests after we see purple cocci on the gram stain.

When it comes to gram positive rods, if they are growing in the aerobic tube think bacillus. If they are only in the anaerobic tube think of clostridium. Listeria is a facultative anaerobe, so it can grow under both conditions.

Slide 38: How about the gram-negative pink-staining organisms. When you see gram negative cocci think Neisseria. The buzz word for this is gram negative diplococci. When it comes to gram negative rods, we typically divide these into the enteric meaning they grow better in anaerobic conditions versus non-enteric that you are more likely to find in lungs and oxygen rich environments.

Slide 39: How about the pathogens that are not going to stain on a standard gram stain? These are organisms such as legionella, chlamydia, rickettsia, and mycobacteria. Classically you need to do a Ziehl Neelsen stain to see mycobacterial organisms. Acid fast bacteria will be red, while nonacid fast bacteria will stain blue/green with the counterstain.

Slide 40: How about diagnosing other pathogens? When it comes to fungi what is really helpful is how they look, do they have hyphae are they branching or not branching and if they are branching do they branch at acute angles. Are there fungal capsules? What is the size of the yeast?

For viruses typically we will use PCR testing or antigen detection. Antigen detection is how many rapid covid tests work. Antibody detection and serology can also be helpful. Virus isolation from culture is very difficult as is direct electron microscopy.

When it comes to parasitology these are typically larger organisms that we look at under the microscope. You can also use antigen detection and serology.

Slide 41: When it comes to empiric antibiotics you have to ask yourself a few questions: what class of pathogens am I treating? If bacterial is this most likely gram positive? Gram negative? Anaerobic? What information can I get to guide my treatment? In other words what test should I order? And lastly, how sick is my patient? How risky would it be if I missed the coverage of a potential pathogen?

Slide 42: IN terms of general indications for antibiotics you can think about it in 3 big buckets. First is prophylaxis. This means that we are trying to prevent infection. Think of surgical prophylaxis. We know that the surgical site gets contaminated by bacteria, so we use these to prevent a subsequent SSI. The next big bucket is empiric antibiotics. You don't know what is causing the infection, but you are treating a specific clinical syndrome. For example, you suspect that a patient has cellulitis. You don't know what is causing it all the time, so you start with empiric vancomycin to cover gram positive organisms. The last one is the directed antibiotic therapy meaning that you know that causative pathogen such as an E coli UTI. Unfortunately, this probably represents the smallest proportion of folks we see in the hospital.

Slide 43: De-escalation Allows initial broad therapy to maximize initial effective therapy Then target/narrow antibiotic therapies after more data returns (microbiology, clinical progress, diagnostic tests). Avoid unintended consequences of extra days of broad therapy.

Slide 44: So, let's talk about cultures as a means of diagnosis. Cultures are really considered the gold standard. This requires sampling of the site of infection prior to therapy. Typically, we say that patients should be off antibiotics for 14 days prior to cultures in order to maximize culture yield. Cultures also allow determination of antimicrobial susceptibility and can help with outbreak investigations so we can compare genetics.

Slide 45: However, there are some key limitations to cultures. It is time and resource intensive. You typically won't have a lot of information for the first couple of days aside from the gram stain and it requires folks in the micro lab to look at plates and perform biochemical tests. That being said, it is very reliant on specimen collection techniques such as blood culture collection and can be positive in the absence of infection such as is the case with contamination with blood culture collections. In addition, it can also be negative when infection is present such as in the case when patients are on antibiotics or when you can contamination or mixed flora.

Slide 46: Practice Question – *Answer C you want to transport the specimens within 2 hours of collection and in leakproof specimen contains and leakproof bags*

Slide 47: So, let's talk a little bit more about contamination of cultures. First and foremost, inadequate specimen collections can lead to confusing results. They make you think is this real. Is it not? The best example is contaminated blood cultures with skin ~~for a~~ organism. More specifically your patient gets 2 sets of Blood culture s and one of them grows coagulase negative staph, which is a known skin colonizer. Is this real? This is why we always ask to collect blood cultures in pairs and avoid drawing blood cultures from indwelling lines. Other examples include urine cultures from an existing foley catheter. Remember each

day a foley catheter is in place you increase the risk of colonization by 5-7%, so you may just be getting the organism that is living in the catheter but not the true pathogen causing infection. The same thing goes with lower respiratory cultures. You have to get a good quality sample.

Slide 48: So, what are some key specimen collection points. First, always use aseptic technique and use appropriate skin prep. Second you always want to get sterile fluid and not specimens that are going to be contaminated from the environment. In other words, superficial swabs are useless. Many times, you may just be culturing what is on the patient's arm. Third, the more volume the better. Often times, there are very few pathogens per mL of fluid so the more you can get the more likely you will be to recover a pathogen. Next, if you have a sample from the OR it is best to send it for both pathology and microbiology so that you can also get an idea of if there is significant inflammation and sometimes you can even see pathogens from those samples. You also want to label the specimens appropriately. Do not send cultures from indwelling devices like drains and foleys due to risk of biofilm formation and colonization Lastly, don't let specimens sit around. If you set a specimen of urine on the counter and let it sit around for several hours at room temperature it is going to grow pathogens even if none existed there previously.

Slide 49: Practice Question- *Answer B. Colonization. MRSA is a very common colonizer of our nasal passages.*

Slide 50: So, what exactly do we mean by infection versus colonization? Human beings are not sterile. We are made up of communities of organisms that was affectionately refer to as the human microbiome. However, clinicians have trouble not treating when they see a positive culture. Clinical presentation is very important. In that same vein going through clinical criteria for diagnosis should lead to testing not testing leading to clinical suspicion.

Slide 51: By just sending off tests, this can lead to overdiagnosis, overtreatment and potentially negative consequences. The one we cite all the time is asymptomatic bacteriuria. Patients get antibiotics and then develop C difficile infection. So, before you send off a diagnostic test you always want to ask yourself, what is the pre-test probability that this patient has an infection and how would this change my management if the test is positive or negative.

Slide 52: So, how do we differentiate between asymptomatic bacteriuria and true cystitis? The big difference is in clinical symptoms. In a true infection a patient will have symptoms versus in asymptomatic bacteriuria there are no symptoms. Note what is not in clinical symptoms for cystitis and that is confusion. There is no study that links confusion to cystitis.

Slide 53: Ok so let's talk a bit about antigen tests. All of you should be very familiar with the test on the bottom left. These identify pathogen-specific proteins. They are particularly useful for viral infections like COVID, HIV, Hep B. It can also be useful for other infections such as cryptococcus, strep pneumoniae and legionella.

Slide 54: Ok so what about serologic tests. These detect the immune response to a pathogen or a prior exposure to a pathogen. For bacterial infections these are not typically useful. However, for viral infections we can look for IgM positivity to indicate early diagnosis or recent exposure. These tests are also important for screening for prior exposure such as for varicella and hepatitis B. Once a serology is positive it is typically life-long.

Slide 55: How about molecular tests? These are increasingly used to diagnose non-culturable pathogens such as norovirus and allow faster identification for pathogens such as MRSA or pertussis. However, they are very sensitive and thus can cause false positives as is the case with C difficile infection. You can have a positive PCR when it really only presents colonization as opposed to a true infection. That's why we often have to combine the PCR with a toxin-based test.

Slide 56: When getting studies on sterile fluid we are looking for inflammatory, chemical, and cellular changes. For example, in synovial fluid or CSF we are looking for the number and types of cells in the fluid to help us know if there is infection. This is typically combined with gram stain and culture.

Slide 57: Practice Question – *Answer A.*

Bonus – typical pathogens to suspect for community onset bacterial meningitis in adults.

Neisseria meningitides (contact and droplet)

H. Influenza

S. Pneumoniae

Listeria monocytogenes

Slide 58: Here's another table that you might want to keep as reference. This gives you an idea of what kind of meningitis you are looking at based on the opening pressure, the glucose, the number and types of white blood cells and the total protein.

Slide 59: Ok let's talk a little bit about antibiotic susceptibility. An antibiotic is a drug that kills or inhibits the growth of microorganisms. If a pathogen is resistant that means that the antibiotic will NOT inhibit the bacterial growth at clinically achievable concentrations, so you shouldn't use that antibiotic to treat that infection. If a pathogen is susceptible to a specific antibiotic that means that it will inhibit bacterial growth at a clinically achievable concentration, so you can use that antibiotic to treat the infection. Lastly, intermediate means that it may not inhibit bacterial growth at typical doses. We typically would not use this antibiotic alone or at standard doses but may use it in conjunction with another antibiotic that the pathogen is susceptible to or at higher doses.

Slide 60: Ok so these are a little tricky and may take a bit of time to fully grasp. The MIC is the lowest concentration that an antibiotic inhibits growth of bacteria. The lower the better. The MBC is the concentration that kills bacteria. We don't use this clinically. A breakpoint is the MIC number that is used to designate between susceptible and resistant and this is set by the CLSI committee. These breakpoints do change over time.

Slide 61: So how do they determine the MIC? Identical doses of bacteria are cultured in wells of liquid media containing progressively lower concentrations of the drug. The minimum inhibitory concentration of the antibiotic is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth.

Slide 62: What are the methods for determining test susceptibility. The first one is broth dilution, which is the MIC testing we just spoke about. The next one is disc diffusion, which are the pictures in the middle. In diagnostic laboratories, the test is performed by inoculating the surface of an agar plate with bacteria isolated from a patient's infection. Antibiotic-containing paper disks are then applied to the agar and the

plate is incubated. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the disk where the bacteria have not grown enough to be visible. This is called a zone of inhibition. The susceptibility of the bacterial isolate to each antibiotic can then be semi-quantified by comparing the size of these zones of inhibition to databases of information on known antibiotic-susceptible, moderately susceptible, and resistant bacteria. In this way, it is possible to identify the most appropriate antibiotic for treating a patient's infection.[1][2] Although the disk diffusion test cannot be used to differentiate bacteriostatic and bactericidal activity, it is less cumbersome than other susceptibility test methods such as broth dilution. The last test on this slide is the E test. You set the reagent test strip with a predefined gradient of antibiotic, covering a continuous concentration range. It is applied to the surface of an agar plate inoculated with the test strain, where there is release of the antimicrobial gradient from the plastic carrier to the agar to form a stable and continuous gradient beneath and in nearby to the strip. After the test, the bacterial growth becomes visible after incubation and a symmetrical inhibition ellipse centered along the strip is seen. The MIC value is read from the scale in terms of µg/mL where the ellipse edge intersects the strip. After the required incubation period, the minimum inhibitory value is read where the edge of the inhibition ellipse intersects the side of the strip.

Slide 63: So, what are resistance mechanisms. These can really be thought of in two broad categories. The first is intrinsic resistance meaning that it is inherited by the organism. All Serratia are intrinsically resistant to cefazolin. You can also get acquired resistance from altered cellular structure and physiology. These can include things like efflux pumps to pump antibiotics out of the cell. Mutations on a penicillin binding protein make antibiotics unable to hook onto them. Beta lactamase production meaning that these enzymes can cut beta lactam rings and make them ineffective and carbapenemase production, which make carbapenems ineffective.

Slide 64: Antibacterial drugs prevent bacterial growth by disrupting the function of a wide variety of molecular targets located within bacteria and at the cell surface. The penicillin's, cephalosporins, and carbapenems all target cell wall synthesis by inhibiting transpeptidases required for peptidoglycan formation and the cross-linking of structures in the cell wall. Metronidazole interacts with DNA and inhibits nucleotide synthesis, leading to cell death. Fluoroquinolones block DNA synthesis by inhibiting DNA gyrase, which is responsible for folding and supercoiling the replicating DNA so that it does not become entangled with itself. The sulfonamides and trimethoprim-sulfamethoxazole (TMP-SMX) inhibit the metabolism of bacteria by inhibiting enzymes needed for the synthesis of folic acid. The peptide antibiotics (eg, vancomycin) form complexes with the peptidoglycans that prevent them from binding to the transpeptidases responsible for cross-linking cell membrane structures into a rigid matrix.

A large number of antibiotics, including the aminoglycosides, macrolides, oxazolidinones, streptogramins, lincosamides, and tetracyclines, interact with bacterial ribosomes to inhibit protein synthesis.[1] In particular, the tetracyclines and glycylcyclines bind to the 30S subunit of the ribosome, inhibit binding of aminoacyl tRNA to the A site of the ribosome, and prevent the transfer of amino acids to newly forming protein chains. Glycylcyclines are a new class of antibiotics developed to overcome resistance to tetracyclines.

Reference

1. Chopra I. Glycylcyclines: third-generation tetracycline antibiotics. *Curr Opin Pharmacol.* 2001;1:464-469.

Slide 65: No notes

Slide 66: Practice Question – *Answer A.*

Bonus: typhi vs. non-typhi (enteritidis)

S. Enteritidis is the most common cause of outbreak-related acute gastro in the US.

Outbreaks associated with many things: exotic pets, most recently pre-cut melons and honey Smacks!

Slide 67: So here is another great reference slide. The timing from ingestion to symptom onset is very important. The most important takeaway from this slide is that if the toxin is causing the infection, then the onset of symptoms is within hours versus bacteria it is typically days.

Slide 68: Practice Question – *Answer C. Scabies The key here is the webs of her fingers. These are typically extremely pruritic.*

Slide 69: Practice Question – *Answer B. Might be as short as 10d, but is typically weeks (4-6)*

He would have to be exposed prior to his hospitalization (by at least 5 days)

Slide 70: Ok so what is the quick and dirty on parasites? A parasite is any organism living within or on another living creature and deriving advantage from doing so while causing a disadvantage to the host. Parasites can be thought of in three big buckets. Multicellular such as lice. Protozoa such as malaria, and worms such as tape worms.

Slide 71: Practice Question- *Answer D*

Slide 72: Prior diseases are rare but something you definitely should be aware of as an IP. Priors can cause transmissible spongiform encephalopathies or TSEs. The infectious protein replicates in the CNS and disrupts neuron functioning. These are rapidly progressive and ultimately fatal. You get a spongiform appearance on the neurologic tissues but there is no inflammatory response. The ways in which to acquire TSEs are from sporadic disease, iatrogenic, familial transmission, or ingestion from infected cows. On the right-hand side, I have infections in humans and in animals. The most famous is Creutzfeldt Jakob disease or CJD.

Slide 73: Practice Question- *Answer C.*

Some people may react to the TST even though they are not infected with M. tuberculosis. The causes of these false-positive reactions may include, but are not limited to, the following:

Previous TB vaccination with the bacille Calmette-Guérin (BCG) vaccine

Infection with nontuberculous mycobacteria (mycobacteria other than M. tuberculosis)

Some people may not react to the TST even though they are infected with M. tuberculosis. The reasons for these false-negative reactions may include, but are not limited to, the following:

Anergy

Recent TB infection (within the past 8 to 10 weeks)

Very young age (younger than 6 months)

Slide 74: Practice Question- *Answer A. Collection of early morning specimens are preferred because of the overnight accumulation of secretions*

Slide 75: So, let's talk a bit about TB. This is an acid-fast bacillus. Clinically it causes pulmonary disease with subacute onset. These folks are sick for weeks to months. The sputum is classically taught to be bloody, and patients often have systemic symptoms such as chills, fevers, and night sweats. TB is diagnosed by sputum culture, PCR DNA probe and CXR. The tuberculin skin test or IGRA is a screening test for latent disease or prior exposure and not for active infection.

Slide 76: No notes

Slide 77: Practice Question – *Answer A*

Slide 78: Ok so what about spirochetes? Lyme is caused by borrelia burgdorfori. It is a tick-borne illness classically thought of in the NE US, but the region of infection is expanding. This classically causes target lesions called erythema migrans, fever, headache, arthralgias. It can also cause CNS disease and associated Bell's palsy and cardiomyopathy. Bell's palsy is manifested by sudden weakness in the muscles on one half of the face. The diagnostics are a bit confusing and often ID clinicians get involved to help. The tick that carries the spirochete is the lxodes scapularis. The treatment is doxycycline but can also be ceftriaxone for CNS involvement.

Slide 79: Another spirochete is syphilis. It is called the great imitator as it can present very similar to a large variety of other diseases, which can sometimes complicate its diagnosis, especially in the later stages. It is sexually transmitted. It causes multiple stages of disease including primary, secondary, early latent, late latent, tabes dorsalis, tertiary, ocular and congenital syphilis. Darkfield microscopy is the classic diagnostic tool, but it is rarely if ever used. We typically use serology IgG plus a confirmatory test. RPR or VDRL titers are used for response to therapy and detection of new exposures. The treatment is good old penicillin.

Slide 80: That's it! We made it through! Please let me know if you have any additional questions!