**PAPER**

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**COURSE: CLINICAL BACTERIOLOGY**

**EXAM: MID-TERM**

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**SECTION A:**

**Case Study**

A previously healthy Sam’s 18-year-old young man presents with a 4-day history of painful swelling on his right thigh. On examination, there is a 3 x 3 cm fluctuant, tender, and erythematous mass. There is no drainage or associated open wound. He has been afebrile, and his vital signs are within normal limits.

**Q1: What is the most common pathogen of this skin infection?**

* ***Staphylococcus aureus***
* Streptococcus pyogenes
* Gram-negative bacteria
* Mixed aerobic and anaerobic bacteria

**Q2:** Pus formation occurs because pathogen produces leukocidins, which kill white blood cells. These purulent skin infections may initially manifest as **folliculitis**, but can lead to **furuncles** or deeper abscesses called **carbuncles**.

**Compare and contrast** folliculitis, furuncles **and** carbuncles **in terms of:**

* **Histological appearance**
* **Characteristic cells involved**
* **Systemic effects**

**Folliculitis** **Histological appearance**

Folliculitis is a skin infection in which inflammation of the hair follicles occurs.

Around the hair follicles i.e. area from where the hair grows, it appears as small red bumps or white headed pimples. You can also see yellow, small pustules that are restricted to hair follicles.

When you are exposed to hot tubs and spas the infection of folliculitis may occurred by the bacterium S.aureus and Pseudomonas aeruginosa.



**Folliculitis Characteristic cells involved**

The characteristics cells involved are skin cells around the hair follicles. They are only limited to moist skin along with hairs and are present as pustules.

**Folliculitis Systemic effects**

They don’t have any systemic effects and are found around hair follicles.

**Furuncle** **Histological appearance**

When bacteria infects one or more hair follicles under the skin and pus filled bumps are formed, it is known as furuncle or boil.

It appears as small, red, tender bumps or nodules which is fluctuant and may become painful with the passage of time and could be present on face, neck, armpits and buttocks.

They are caused by S.aureus and S. pyogenes under the skin which involves the sebaceous glands.



**Furuncle** **Characteristic cells involved**

The characteristic cells involved are hair follicles which may extend to subcutaneous tissue and sebaceous glands.

**Furuncle** **Systemic effects**

They are tender, painful but the person is systemically well and antibiotic therapy is only needed when the infection is spreading systemically.

**Carbuncles** **Histological appearance**

When a group of boils are present under the skin and it cause an infection, known as carbuncles.

It appears as large, red nodules in which pus may occurs.

It is also caused by S.aureus and S. pyogenes below the skin and is most severe form of furuncles.



**Carbuncles** **Characteristic cells involved**

The characteristics cells involved are deeper layers of skin and subcutaneous fat tissues.

**Carbuncles** **Systemic effects**

They are very painful and can spread systemically to other parts of the body as it is similar to furuncles but are severe from them.

**Q3:** Observing that Sam’s wound is purulent, the doctor tells him that he probably has a bacterial infection. Technician takes a sample from the lesion to send for laboratory analysis, but because it is Friday, she does not expect to receive the results until the following Monday. In the meantime, he prescribes an over-the-counter topical antibiotic ointment. He tells Sam to keep the wound clean and apply a new bandage with the ointment at least twice per day.

**How would the lab technician determine if the infection is staphylococcal or streptococcal? Suggest several specific methods?**

The antibiotic ointment which was prescribed by the doctor for the treatment of the wound seems to have no effect on the patient. During the weekend, the technician may have perform different tests to find out that the infection may be occurred by one of the bacterium i.e. staphylococcal or streptococcal or might be by both of them. The test performed for the presence of streptococcus aureus was passive agglutination test through which it was confirmed that S.aureus was present in the wound and for the confirmation of S.pyogenes latex agglutination test and bacitracin susceptibility tests were done which also confirms the presence of staphylococcus pyogenes.

As we know that many strains of staphylococcal are resistant to antibiotics so antimicrobial susceptibility test was also performed for the identification of the staphylococci.

Beside that catalase test which is positive for staphylococci and negative for streptococci could also be done. Coagulase test which is very specific and positive for S.aureus could also be done, and negative for other staphylococcal species. DNAase test can also be performed which is positive for staphylococcus aureus.

**SECTION B:**

* Imagine that you are working in hospital as Microbiologist during coronavirus outbreak
* **How can you classify this novel coronavirus (2019-nCoV)?**
* **What would be the Biosafety Level?**
* **What are the laboratory biosafety Safety Practices Associated with novel coronavirus (2019-nCoV)?**
* **What would be appropriate disinfectants?**
* **What do you know about Packaging and shipment process**?

**How would I classify this novel coronavirus (2019-nCoV?)**

As we all know that the novel coronavirus (2019n-CoV) can be transfer from one human to another and have the potential to cause public panic and social disruption. It can cause high mortality in immunocompromised peoples and can also infect other humans as well. Therefore the viral cultures and isolates of novel coronavirus (2019n-CoV) are classified as category “A, infectious substances affecting humans” while the patient specimens from suspected and confirmed cases of novel coronavirus (2019n-CoV) are classified as “category B biological substances”.

**Biosafety Level**

All the procedures related to novel coronavirus (2019n-CoV) should be perform in strict lab conditions and the initial processing of all the specimens related to novel coronavirus (2019n-CoV) should always be performed in a biosafety cabinet.

Propagative work i.e. isolation, culturing and neutralization of a novel coronavirus (2019n-CoV) should be performed in biosafety-level 3 labs with inward directional flow laboratories and the non-propagative work such as sequencing, nucleic-acid amplification test, polymerase chain reaction etc. should be performed in bio-safety level 2. Remember that proper protection i.e. wearing personal protective equipment’s (PPE), should be followed in each and every step in both propagative and non-propagative works.

Beside that disinfectants should be used before performing any experiment or test against novel coronavirus (2019n-CoV) to sanitize instruments, working place and objects. So, that area, instrument and object should not be effected by the virus.

**Laboratory biosafety Safety Practices Associated with novel coronavirus (2019-nCoV)**

* The laboratory in which you are workingshould ensure that it is safe to perform tests with appropriate risk control measures.
* When handling a specimen for testing and other procedures, good microbiological practices and procedures (GMPP) should be followed.
* The handling of suspected or confirmed cases of novel coronavirus (2019n-CoV) for further testing should follow local guidelines for processing potentially infectious material.
* Non propagative diagnostics laboratory work that include NAAT, PCR of suspected and confirmed cases with novel coronavirus (2019n-CoV) should be conducted by adopting practices and procedures of core requirements in BSL-2.
* Handling of live contaminated virus such as novel coronavirus (2019n-CoV) should be done by properly trained and competent personal laboratories meeting the requirements of BSL-3.
* All the initial processes of the specimen should take place in biosafety cabinet or primary containment device.
* Appropriate disinfection should be used against enveloped viruses, within due dates after preparing the solution.
* Appropriate PPE should be worn by all the laboratory workers that are handling the novel coronavirus (2019n-CoV) specimens.
* Patient specimens with suspected or confirmed cases would be transported as category B biological substance and viral cultures and isolates would be transported as category A.

**Appropriate disinfectants**

As we all know that very little is known about the novel coronavirus (2019n-CoV) and we also know that some of its genetic characteristics matches with Severe acute respiratory syndrome-related coronavirus (SARS-CoV) and Middle East respiratory syndrome-related coronavirus (MERS-CoV) which shows that the novel coronavirus (2019n-CoV) is susceptible to disinfectants.

The appropriate disinfectants against novel coronavirus (2019n-CoV) are bleach for general surface and blood spills on a surface, 70% ethanol, 0.5% hydrogen peroxide, peracetic acid, iodophors, biguanides, triamines, sodium hypochlorite and phenolic compounds, quaternary ammonium compounds. Other biocidal compounds which are less effective, used against novel coronavirus (2019n-CoV) are 0.05-0.2% benzalkonium chloride or 0.02% chlorhexidine.

**Packaging and shipment process**

Packaging is the process of wrapping to protect materials. In microbiology those materials which are transported between laboratories for different purposes such as experimentation, research work, cultivation etc. to minimize the potential for breakage or a spill, to protect the environment from contamination and to protect life of living organisms are placed or wrapped in a container, is called packaging.

Shipment is the transportation of materials from one area to another. In microbiology shipment is the process or transporting specimens within national boundaries for experimentation, research work, cultivation, sequencing, testing etc. following the rules and regulations of that particular country in which you’re residing.

**SECTION C:**

**What is the relationship between normal flora and human beings? Enlist and briefly describe the pathogens which has both positive and negative effect on human being?**

**Relationship between normal flora and human beings**

As we all know that microbes are everywhere in the environment and they have several important roles in regulating different processes in the environment such as carbon fixation, nitrogen cycling, phosphorus and iron cycling, mineral neutralization etc. Same like that they live in and on the humans and helps in different processes such as producing enzymes that helps in breaking food molecules, converting one compound into another, making proteins, helping in metabolism, synthesizing vitamins, synthesizing enzymes, regulate appetite, regulate blood sugar level, regulate hormone metabolism, synthesizing neurotransmitters, produce toxins etc.

We can say that humans needs microbes and many microbes need human for their survival as they provide the specific environment that is needed for their survival. Different microbes reside on different places in the human body and on the human body and they have adapted these conditions for their survival and growth or we can say that human and microbes rely on each other for their survival and growth.

Normal flora are the microorganisms that live in and on human beings. Following are some of the normal flora organisms that survive on different regions of human body:

**Skin normal flora**

Microbes that live in and on the surface of skin are known as skin normal flora. Skin is that part of the human body, which is in direct contact with the external environment and due to this reason, great variety of microorganisms are present on human skin. Thousands of different species of bacteria, fungi, viruses live on skin, most of them are harmless and beneficial to humans.

Microorganisms that are present on the upper parts of epidermis and around the hair follicles are:

* Staphylococcus
* Micrococcus
* Corynebacterium
* Brevibacterium
* Dermabacter
* Malasezzia etc.

S. epidermidis is a major inhabitant of the skin, anaerobic diphtheroids are present in areas that are rich in sebaceous glands.

The benefits of these microbes are, they prevent pathogenic microbes from colonizing on skin surface by secreting chemical agents against them and by activating the skin immune system.

**Oral/Mouth micro flora**

Microbes that are normally present in mouth are known as oral/mouth normal flora. About 600-700 species of different bacteria, fungi and other organisms are present in the mouth. Species that are present in mouth micro biota are:

* Streptococcus
* Actinobacteria
* Bacteroidetes
* Chlamydiae
* Chloroflexi
* Firmicutes
* Proteobacteria etc.

These microbes gets nutrients from the host for their growth and survival but they also contributes to host nutrition by synthesizing vitamins. They also contributes in immunity of the host by producing antibodies against pathogenic organisms.

**Quorum sensing**

The association of two or more species is known as quorum sensing.

**Microbes in upper respiratory tract**

Microbes that lives in the respiratory tract are known as respiratory micro biota. Respiratory tract are the initial site of colonization for pathogens such as:

* Neisseria meningitides
* C. diphtheria
* Bordetella pertussis
* Proteobacteria
* Crenarchaeota
* Neisseria etc.

**Microbes in lower respiratory tract**

Lower respiratory tract is sterile and bacteria can’t reach these regions.

If they reach these regions they counter the host defense system and alveolar macrophages are activated.

**Intestinal flora**

Microorganisms that lives in the intestine are known as the intestinal flora. The micro flora of intestine is very complex containing over 400 bacterial species. Intestinal flora is also known as gut flora or gut micro biota. Most of the intestinal micro flora are present in colon and that is:

* Peptostreptococcussp.
* Eubacterium sp.
* Lactobacillus sp. and
* Clostridium sp. etc.
* **Duodenum** contains some gram-positive cocci and rods
* **Jejunum** contains Enterococcus faecalis, lactobacilli, diphtheroids, and Candida albicans.
* **Ileum** has microbial community similar as colon.

They helps us in regulating immunity, synthesizing vitamins, synthesizing enzymes, regulate appetite, regulate blood sugar level, regulate hormone metabolism, synthesize neurotransmitters, control pathogens and increase mineral bioavailability.

**Nail Flora**

Microbes living in nails are known as nail flora and have similar organisms as skin flora. Dust particles containing fungi are also present due to contact of nails with extraneous sources. It includes:

* Pseudomonas
* Staphylococcus
* Klebsiella
* Aspergillus
* Candida albicans
* Cladosporium, and Mucor etc.

**Conjunctival Flora**

Microbes that reside in the conjunctiva are known as conjunctival flora. The conjunctival flora is sparse and may contain:

* Corynebacteria,
* Neisseriae
* Moraxellae
* Staphylococci
* Streptococci and
* Haemophilus parainfluenzae etc.

Lysozyme present in tears play an important role in in controlling the bacteria by interfering with their cell wall formation.

**Pathogens having both positive and negative effects on human being**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No.** | **Name of a**  **pathogen** | **Positive effect** | **Negative Effect** |
| **1.** | **Bacillus subtilis** | * Symbiotic microorganism present on skin that produces a bacitracin toxin that helps in fighting with pathogens * Preservation of food * Cancer treatment | * Food poisoning * Ropiness |
| **2.** | **Streptococcus mutants** | * Produced bacteriocin that inhibits growth of other bacteria. | * Dental caries |
| **3.** | **Gut micro biome:**  **Clostridium**  **Lactobacillus**  **C. diphtheria** | * Helps in nutrition * Immunity * Biosynthesis of vitamin K * Conversion of bile acids | * Peritonitis * Abscesses * Diarrhea * Distention |