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M.Sc. [Microbiology]

III - Semester

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MEDICAL MICROBIOLOGY

Author :

Dr. S. Rajan
Assistant Professor
PG and Research Department of Microbiology
M. R. Government Arts College
Mannargudi -614 001

Reviewer
Dr.G.Selvakumar
Assistant professor of Microbiology
Directorate of Distance Education
Alagappa University
Karaikudi

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BLOCK I
MEDICAL MICROBIOLOGY – AN INTRODUCTION

INTRODUCTION

Microbiology is the new field which has emerged from basic biological sciences. Development in microbiology started in the late 19th century. One of the most important health oriented field in microbiology is Medical Microbiology. It is also called as physician oriented microbiology. Medical microbiology deals with nature of causative agent, its pathogenesis, symptoms of the disease, epidemiology, laboratory diagnosis, treatment and prevention of microbial diseases. Diagnostic microbiology is a part of medical microbiology which deals with the sample collection, transport and microscopic analysis, cultivation of causative agents and characterization of etiological agents of disease. Medical microbiology includes bacteriology, virology, mycology and parasitology.

UNIT I - LABORATORY MANAGEMENT

Structure

1.1. INTRODUCTION

Microorganisms are the major element in the microbiology laboratory. Laboratory should be properly managed for better recovery of microorganisms from clinical sample. Improper management may leads to contamination in laboratory, which intern leads to infection in laboratory staff. Waste from laboratory also properly managed otherwise ecosystem adjacent to the laboratory also affected. Adherence to standard microbiological techniques and using facilities suitable to the risk level of the pathogen helps to protect the researcher from laboratory-acquired infections. This chapter explains safety in containment laboratory.

1.2. OBJECTIVES

After reading this unit, learners will understand

- Safety of containment laboratory.
- Types of containment
- Biohazards.
- Classification of pathogenic microorganisms with reference to laboratory.
- Biosafety levels.
- Good microbiological techniques
- Decontamination
- Biohazard waste disposal.
- Samples required for clinical examination
- Methods of sample transport.

1.3. SAFETY IN CONTAINMENT LABORATORY

Safety is a concept that includes all measures and practices taken to preserve the life, health and bodily integrity of individuals. The actions of keeping something harmful under control or within limits are also called **containment.** Containment procedures are essential for the maintenance of safety. **Biocontainment** is the safety practices used in laboratory to prevent unintended infections. It is a component o bio-risk management. Objective of biocontainment is to confine an infectious organism or toxin thereby reducing the chance of exposure to laboratory workers or persons of outside of the laboratory. The term biocontainment was coined in 1985.

1.3.1 Types of containment

There are two types of containments. They are Biological containment and Physical containment. **Biological containment** (BC) is any combination of vector and host which is to provide safety. Biological containment must be chosen or constructed to limit the infectivity of vector to specific hosts and control the host-vector survival in the environment. **Physical Containment** (PC) helps to confine the pathogenic organisms being handled and prevent exposure to personnels involved in lab activities. Physical containment is achieved by laboratory practices, containment equipment and special laboratory design.

On the basis of microbiology laboratory, biocontainment are divided into two. They are Primary containment and Secondary containment. Primary containment offers protection to personnel and immediate laboratory environment whereas secondary containment offers protection to the environment outside the laboratory. Primary containment adhering to standard microbiological practices and techniques and awareness of potential hazards. This includes biological safety cabinets and enclosed containers. Secondary containment is concerned with proper design of the laboratory facility which helps in protecting personnel inside the facility and also prevents the release of pathogenic organisms outside the facility.

Laboratory Facility designs are of three types. They are Basic Laboratory (for Risk Group I and II), Containment Laboratory (for Risk Group III) and Maximum Containment Laboratory (for Risk Group IV).

1.3.2. Bio hazards

Biohazard means biological substance possesses threat to living animals and human. Hazards related to bio research can be classified into two categories. They are hazards related with the pathogen or human/animal cells being used in research and hazards related with the procedures and practices followed in the lab.

1.3.3. Routes of entry for pathogen

The probable routes of entry of pathogens are inhalation of infectious aerosols, contact of the agent with the skin, eyes or mucous membrane, inoculation by contaminated sharps, bites from infected animals or contact with their body fluids and ingestion of infectious agent through mouth pipetting or contaminated hands.

1.3.4. Aerosols

Aerosols are generated during research activities. It can spread easily and remain suspended in the laboratory atmosphere for a long time. They possess a serious hazard to the person performing the task and also to others who are exposed to the air from the laboratory. Aerosols can be generated during the following activities like pipetting, blending, centrifugation, use of sonicators and vortex mixers. These respirable size particles when inhaled are retained in the lungs and can cause infection to the person.

The risk from the pathogen handled depends on the following factors. They are capability to cause infection in the host and the severity of the same, preventive measures and treatment available, route of entry, infective dose level, stability in the environment and the range of cells/strains that can act as a host.

1.3.5. Classification of pathogenic microorganisms

Based on the above risk factors the microorganisms are classified into four risk groups. They are as follows

Risk group I - A pathogen that is unlikely to cause any disease in humans or animals.

Risk group II - A pathogen that can cause disease in humans or animals but
is unlikely to be a serious hazard. Effective treatment and preventive measures are available and the risk of spread of infection is limited.
Examples : • Bacteria- V*ibrio cholera;* • Fungus- *Aspergillus fumigatus,* • Parasite- *P.falciparum;* • Virus - *Mumps virus.*

Risk group III - A pathogen that can cause serious human or animal disease, but does not ordinarily spread from one infected person to another. Effective treatment and preventive measures are available. Example • Bacteria - *Clostridium botulium;* • Fungus - *Histoplasma capsulatum;* • Parasite- *Schisistosoma mansomi;* • Virus - *Foot-and-Mouth disease virus.*

Risk group IV - A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. Examples are *Korean hemorrhagic fever, Omsk hemorrhagic fever and Central European Encephalitis viruses*

1.3.6. Biosafety levels

It consists of a combination of laboratory practices, equipment and facilities suitable to the procedures being performed and hazards of the pathogen. The four biosafety levels correspond to four risk groups. A lower risk group can be assigned a higher biosafety level.

Biosafety level I is suitable for teaching laboratories and for facilities in which work is done with defined and characterized strains of agents not known to cause any disease. Good microbiological techniques (GMT) to be followed to maintain laboratory safety.

Biosafety Level II is applicable to facilities in which work is done with indigenous moderate-risk agents present in the community and associated with human disease of varying severity. BSL II is appropriate when work is done with any human derived blood, body fluids, tissues, or primary human cell lines, in which presence of an infectious agent

may be unknown BSL II requires GMT, Use of personal protective equipment, Use of BSC and use of autoclaves.

Biosafety level III is applicable to facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences. BSL III requires in addition to that of BSL II requirements. They are special clothing, directional airflow, controlled access, double door entry/Anteroom and supervision.

Biosafety level IV is applicable to work with dangerous and exotic agents which pose a high individual risk of life-threatening disease. BSL IV requires in addition to BSL III requirements are as follows. Positive pressure personnel suits, Strictly limited access, Double ended autoclave, Class III BSC, Airlock with shower and Supervision.

1.3.7. Good microbiological techniques (GMT)

• Specimen containers must be correctly labeled for easy identification.
• Use containers (autoclavable) while transporting specimens to contain spill.
• Specimen containers received from external agencies must be opened in the biosafety cabinet.
• Use mechanical pipettes.
• Open flame must not be used in Biosafety cabinet as it can distort the air flow pattern and damage the filters.
• Always use disposable gloves. Do not touch mouth, eyes and face with contaminated hands.
• Food and drink must not be stored or consumed in the laboratory.
• Glassware must be replaced with plasticware wherever possible.
• Sharps(e.g., needle sticks, glass) must be avoided wherever possible as it can transmit blood borne pathogens in case of injury.
• Use engineered sharp-safety devices when syringes and needles are necessary.
• Needles must not be recapped, to prevent needle stick injury.
• Puncture-proof containers fitted with covers must be used for disposing sharps.
• Tubes and specimen containers must always be securely capped (screw-capped if possible) for centrifugation.
• Refer to manufacturer's instructions before operating equipments.
• Work area must be decontaminated with a suitable disinfectant at the end of the work.
• Hands must be thoroughly washed before leaving the lab.

1.3.8. Personal protective equipment

• Personal protective equipment acts as a barrier to minimize the risk of exposure to aerosols, splashes and other injuries.
• Personal protective equipment must be selected on the basis of the risks involved in the task performed.
• Lab coat, safety glasses and toe covered footwear is a minimum requirement while working in the lab.

• Face shield must be used if there is any risk of splashing of infectious materials.
• Gloves must be worn for all procedures that may involve direct contact with blood, infectious materials, or infected animals.
• Gloves must be removed aseptically and autoclaved with other laboratory wastes before disposal.
• If re-usable gloves are used, on removal they must be cleaned and disinfected before re-use.
• Lab coats and other personal protective equipment used must not be used outside the laboratory.

1.3.9. Biosafety cabinets (BSC)

Biological safety cabinets provide containment of infectious aerosols generated during the laboratory procedures. Three types of BSCs are used in microbiological laboratories. These are Class I, Class II and Class III. Class I BSC - Offers protection to laboratory personnel and to the laboratory environment. It doesn't protect the samples from external contamination. Class II BSC provides protection to the samples in the cabinet from external contamination in addition to personnel and laboratory environment protection. Class III BSC - Provides the maximum attainable level of protection to personnel and the environment. The following factors reduce the efficiency of the BSC. They are poor location, room air currents, decreased airflow, leakage in HEPA filters, working with raised sashes, overcrowding the work surface and improper user methodology.

1.3.10. Emergency measures

In case of exposure to bio samples. Laboratory should follow the following.
• Remove the contaminated clothing.
• Wash the skin thoroughly with soap and water.
• In case of eye contact flush the eyes with water.
• Report the exposure to the Lab in charge.
• Get medical attention immediately.

1.3.11. Decontamination

• Decontamination renders an item (work bench, equipment, etc.) safe to handle by reducing the number of organisms to below the threshold infectious dose level such that transmission is unlikely to occur.
• Decontamination requirements will depend on the experimental work and the nature of the infectious agent handled.
• Decontamination is usually accomplished by steam sterilization or autoclaving.
• Sterilization and disinfection are different forms of decontamination.

Sterilisation - Sterilisation makes an item free from all living microorganisms and viruses. The process of sterilization can be accomplished by applying heat.

Disinfection – it is not as effective as sterilization, as some organisms such as bacterial endospores may survive. A disinfectant is a chemical or mixture of chemicals used to kill microorganisms, but not spores. They

are usually applied to inanimate surfaces or objects. Sodium hypochlorite and formaldehyde are the disinfectants recommended for general laboratory use.

• For special purposes phenolic compounds, alcohols, iodine etc., can be used effectively.

1.3.12. Biohazard waste disposal

Biohazard waste generated in laboratories must be segregated into the following:

• Non-contaminated general waste
• Sharps-needles, glass pieces, etc
• Contaminated material for autoclaving and recycling
• Contaminated material for incineration
• Biohazard waste for autoclaving must be collected in red plastic bags and those for incineration in yellow non chlorinated plastic bags.

Check your progress

1. 1.How aerosols are generated
1.2. Is Biohazards cause any damage to human
1.3. Is Safety is needed in Microbiology laboratory
1.4. What is Sterilization? Is it different from Disinfection?
1.5.What are the purposes of using Biologicalsafety Cabinet.

• Biohazard waste of human and animal origin must be incinerated.

1.4.COLLECTION AND TRANSPORT OF CLINICAL SAMPLES

1.4.1. Introduction

Specimen is a part of body fluid which actually represents infectious condition of the body. A **specimen** represents a quantity of human material that is tested, examined or studied to determine the presence or absence of particular microorganisms. Several guidelines on the safety precautions to be used in selection, collection and handling of the specimen. Highly essential feature of the sample collection is selecting the proper specimen and collecting adequate sample for examination. It is used for the confirmation that a microorganism is responsible for the infectious disease process. A poorly collected specimen results in, failure to recover important microorganisms lead to incorrect or harmful therapy.

1.4.2. Characters of Good Specimen

- The specimen must be material from the actual infection site and must be collected with a minimum of contamination from adjacent tissues, organs or secretions.
- Optimal times and total number of specimens for collection must be established for the best chance of recovery of causative microorganisms.

- A sufficient quantity of specimen must be obtained to perform the culture techniques.
- Appropriate collection devices, specimen containers and culture media must be used to ensure proper isolation.
- Whenever possible, specimen should be obtained before the administration of antibiotics.
- The specimen container must be properly labeled.

The following information's should be transcribed along the specimen

- Name of the patient
- Hospital name
- Age of the patient
- Specimen type
- Clinical diagnosis
- Possible isolation
- Date and time of collection.

1.4.3. Collection and transport of specimens from skin and soft tissues

Skin harbours millions of microorganisms as normal flora. Normal flora becomes opportunistic pathogen during unfavourable condition. Some of the skin infections are folliculitis, furuncles, scalded skin syndrome, impetigo, cellulitis etc.,. To identify the causative agents of these infections the following methods of specimen collections are required.

Specimen

- Tissue obtained during surgical procedure
- Aspirated material from an abscess or deep wound
- Pus or exudates obtained from an infected site during surgery can be aspirated into the syringe.
- Skin swab
- Skin scrapings
- Nail clippings
- Hair.
- Before sample collection skin surface should be disinfected with 70% alcohol.

Transporting

- Stuarts or Amies transport medium is recommended for swabs.
- Aspirated material should be injected into a anaerobic vial that exclude oxygen.
- Tissue samples for culture should be delivered promptly to the laboratory in sterile container.

1.4.4. Collection, transport of sample from eye

Common infections of eye are conjuctivities, style and dacryoadenitis. Collection of specimens should be done by trained personnel only. Use a dry sterile cotton swab for collection eye swab.

Eye swab is transfered to laboratory immediately with a duly filled request form at the earliest.

1.4.5. Sample collection and transport from upper respiratory tract

Microbial etiology of upper respiratory track is multifactorial. It causes acute infective rhinitis, common cold, pharyngitis. Upper respiratory track is a site for localized infections. It includes infection of throat, middle ear and sinuses. The following specimens are collected during infections of upper respiratory track. They are Nasopharyngeal swab, Sodium alginate throat swab for pertusis, Sinus washings, Surgical biopsy, Swab of posterior pharynx, Swab of tonsil, Pernasal swab and nasopharyngeal aspirates.

Nasopharyngeal aspirates - Gently pass a sterile catheter through one tonsil as far as the nasopharynx. Attach a sterile syringe to the catheterand aspirate the specimen of mucopus. Dispense the specimen into a sterile container.

Nasopharyngeal swab or throat swab - Bright light from over the shoulder of the patient should be focused in to the oral cavity. The patient is instructed to open the mouth at 'aah' position and breathe deeply. The tongue is gently depressed with tongue blade to visualize the tonsillar fossae and posterior pharynx. The swab is extended between tonsillar pillars and behind the vulva. The tonsillar areas and posterior pharynx should be firmly rubbed with the swab. After collection the cotton swabs are placed into sterile Cary Blair transport media to prevent desiccation during transit to the laboratory.

1.4.6. Collection, Transport of Specimen from Ear

Otitis media is a major infection of middle ear. It is caused by bacteria, virus and Mycoplasmas. This infection is spread from throat through Eustachian tube.

- Cotton swab is used to collect ear sample.
- Collect the swab in to a sterile leak proof container.
- Antral washings also collected for the isolation of anaerobes.
- Transfer samples to laboratory as soon as possible If delay is anticipated send the specimen in Stuart's transport medium

1.4.7. Sample collection from lower respiratory track

Lung is considered as a lower respiratory track (LRT). LRT samples are obtained through Needle Aspiration Method. In this method, a needle is inserted in to the throat region and collects sputum specimen. Needle aspiration includes Transtracheal Aspiration and lung aspiration. Other methods are bronchial washing and blood collection. Early morning sputum is most preferred sample from LRT. It contains pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. For collection sterile wide mouthed jar with a tightly firmed screw cap lid can be used.

Transporting

If AFB suspected sputum is transported or stored by adding Cetyl Pyridinium Chloride - Sodium Chloride. (CPC-NaCl) It digests sputum and prevents the over growth of other pathogens.

1.4.8. Sample collection and transport from gastro intestinal system

Gastrointestinal system starts with mouth and ends in anus. Oralcavity, oesophagus, stomach, intestine are the parts of GI system. Ulcer, diarrhea and dysentery are the major symptoms associated with microbial infection.

Stool or rectal swab or gastric aspirate or gastric biopsies are collected during GI Infection.

Specimen Collection - The collection of diarrhoeal stool is not difficult. In cases of diarrhoea, stool specimen should be collected in clean, wide mouthed containers that can be covered with tight fitting lid. In some instances, collection of rectal swab rather than stool specimen maybe necessary, particularly in neonates or in severely debilitated adults. Gastric aspirate is collected with the help of Intubation. Sample collection from the hollow tube is called **Intubation.** Long sterile tube is attached with syringe and the tube is either swallowed or passed through a nostril into the patient stomach. Specimens are withdrawn periodically. The most common intubation tube is Levin Tube.

Transport - The specimen should be transported as early as possible. Don't refrigerate the stool specimen if possible because certain species of *Shigella species* are susceptible to cooling and drying.

1.4.9. Sample collection and transport from urinary track

Urinary track consists of kidney, ureter, bladder and urethra. UTI by bacteria is acquired by the ascending route from the urethra to the bladder. Urethritis, cystitis and pyelonephritis are the few bacterial UTI infections**.**

Sample Collection

Mid stream urine - It is collected in sterile, dry, wide necked, leak proof container. About 20ml of sample should be collected. Clean catch method is used to collect mid stream urine, first voided urine is not collected because it is contaminated with microbes from lower portion of the urethra. If immediate delivery to the laboratory is not possible, the urine should be refrigerated at 4^0C. If the delivery of more than 1 hour is anticipated, boric acid should be added to the urine. Specimen containing boric acid need not be refrigerated.

Cathetral urine - A catheter is a tubular instrument used for withdrawing fluids from the body cavity. Three types of catheter may be

used. They are Hard catheter, French catheter and Foely catheter - multiple samples may be collected at a time.

Suprapubic aspiration -Suprapubic aspiration is performed only in neonates, small children's and occasionally for adults with clinically suspected UTI those who fail to establish diagnosis. This technique is best performed when the bladder is full. The suprapubic skin overlaying the urinary bladder is disinfected and tap is made. In the immediate site where the tap is to be made, about 1ml of anesthetic solution was injected subcutaneously. With the point of a sharply tapered surgical blade, make a small lance wound incision through the epidermis. Through this wound gently extend an 18-guage needle into the urinary bladder and aspirate 10ml of urine into the syringe. All the samples are transported immediately to the laboratory.

1.4.10. Collection and transport of blood sample

Presence of bacteria in blood is called bacteremia. Septicemia, pyaemia, fungemia, viremia, endotoxemia are associated with blood.

Sample Collection

Blood collection was performed by Needle aspiration procedure. To reduce the contamination during vein puncture, the following method should be followed.

Wash with green soap

Rinse with clean water

Apply 1-2% tincture of iodine and allow to dry for 1-2 minutes.

Remove iodine with 70% alcohol.

Blood should be collected before anti microbial treatment has been started and at the time when the patients temperature begins to rise.

Blood for culture should be taken by vein puncture.

10-20 ml of blood collected from adult

1-2.4 ml collected from young infants

2.4- 5 ml collected from old infants.

Two-blood culture should be performed from each patient to confirm the causative agent.

Blood Collection -Venipuncture Procedure

Blood collection tube is labeled with the patient's particulars. Tourniquet is put on the patient about 3-4' above the venipuncture site. Instruct patient to form a fist so veins are more prominent. After finding the vein, clean the venipuncture site with alcohol using circular motion. Allow the area to dry. Assemble needle with syringe. Remove cap from needle. Use thumb to draw skin tight about 1-2" below the venipuncture site. Hold the skin tight through. Insert the needle, bevel side up, into the vein. Push the tube completely onto the needle. Blood should begin to flow into the tube until vacuum tourniquet is exhausted. Release the tourniquet. After opening the patient's hand, place dry gauge over the venipuncture site and slowly remove the needle. Apply mild pressure to the pad. Apply bandage or continue applying mild pressure until bleeding has stopped. Properly dispose of all contaminates supplies in sharp / biohazard container. Place request form and specimen in biohazard bag

1.4.11. Collection and transport of sample from genital track

Urethritis, genital ulcers, vaginal discharge, genital warts, PID is the symptomatic conditions genital system. It may be due to sexually transmitted diseases and other microbial infections.

Specimen Collection

Female - Catheter aspirate, Aminocentesis fluid, Bartholin gland aspirate, Transcervical aspirate, Fallopian aspirate, Swab of genital ulcer, Urethral discharge, Swab of posterior vagina

Male - Urethral swab, Swab of genital ulcer, Penial discharge, Prostetic secretions. For cases like Syphilis blood samples is collected and perform serological techniques.

Urethral specimen collection - It is collected Urethritis in male. Patient should not have passed urine 2 hours before the specimen is collected. Cleans round the urethral opening using a sterile swab moisten with sterile normal saline. Gently massage the urethra from above downwards and collect a sample of pus with a sterile bacteriological loop or sterile swab or at least directly onto a clean slide.

Cervical specimens collection -Moisten a vaginal speculum with sterile warm water and insert it into the vagina. Do not use antiseptics or gynaecological exploration cream. Pass a sterile swab into the endocervical canal gently rotate the swab to obtain a specimen. Make a smear on a slide for Gram staining and another for Chlamydia if facilities available.

Vaginal specimens - Samples may be collected from the posterior fornix of the vagina using a sterile swab. Make a smear on a slide for gram staining.

Specimens from genital ulcers -Genital ulcers can be caused by herpes virus, *Treponema pallidum, Haemophilus ducreyi, Calymmatobacterium granuulomatis and Chalmydia trachomatis*. Protective gloves should be worn. Squeeze the ulcer between two fingers and clean the ulcer surface with saline. Remove any crusts if present. Wipe away the first few drops of blood. Collect a sample of serous exudates by touching a clean glass slide to the lesion. Place a clean cover slip. The specimen may be aspirated from the lesion or the enlarged lymph node using a sterile syringe. Examine immediately under a dark-field microscope.

1.4.12. Collection of samples from CSF

Cerebrospinal Fluid (CSF) is collected from patients with clinically suspected infection of the Central Nervous System (CNS). CSF is used for general laboratory investigation (sugar, protein & cells) & for etiological investigation to identify the etiological agent.

Specimen

Lumbar puncture and Brain abscess

The chance of recovery increases with the volume of the specimen.

Suggested volumes are

1ml for bacterial culture

2ml for fungus
Don't refrigerate the specimen

Lumbar puncture procedure

Gather all materials required for CSF collection. Wear surgical mask and sterile latex gloves that are impermeable to liquids. Label the collection tubes with appropriate information. Ensure that the patient is kept motionless during the lumbar puncture. Disinfect the skin along a line drawn between the crests of the two ilia with 70% alcohol and povidone-iodine to clean the surface and remove debris and oils. Allow to dry completely. Position the spinal needle between the 2 vertebral spines at the L4-L5 level and introduce into the skin with the bevel of the needle facing up. Accurate placement of the needle is rewarded by a flow of fluid, which normally is clear and colorless. Collect CSF into sterile screw-cap tubes. Withdraw the needle and cover the insertion site with an adhesive bandage. Discard the needle in a puncture-resistant, autoclavable discard container.

Transport the CSF to a microbiology laboratory within 1 hour for culture and analysis.

1.4.13. TRANSPORT AND STORAGE OF SPECIMENS FOR VIROLOGY

a) Specimens for isolation of infectious virus should be transported to the laboratory without delay in order to maximize the recovery of the infectious agent.

b) When short delays are anticipated due to unavoidable circumstances or during transport over long distances optimum preservation of infectivity is obtained by placing the container in melting ice or refrigerating the sample at $4^{o}C$.

c) In general freezing the specimen should be avoided but if long delays are inevitable (>48 hours) specimen should be frozen at -70oC, not -20oC.

d) In the hospital environment, taking specimens at night should be avoided. But when this practice is unavoidable, it is important to place the container in the fridge rather than the freezer compartment.

e) Transport from a single point of origin of a large number of specimens inside a single plastic bag should be discouraged. Because, a single leaking container can lead to wastage of all specimens and potential source of infection to exposing staff in such situations. Most satisfactory transport system is to provide racks to keep the blood /specimen -bottles upright and store the racks inside a sturdy insulated container provided with a carrying handle.

f) Transport of pathological specimens by air is governed by strict guidelines laid down by the International Air Transport Association (IATA). It is the responsibility of the shipper to adhere to current IATA regulations as the penalties for contravening IATA regulations are severe.

1.5. LET US SUM UP

Safety in Containment Laboratory

NOTES

Medical microbiology is a branch of microbiology which deals with diseases, its causative agents, pathogenesis, symptoms, laboratory diagnosis, prevention and treatment of diseases.

Microbiologists are those who practicing isolation and identification of microorganisms are called medical or clinical microbiologists.

Microbiology laboratory involves collection of sample, transport and processing of clinical and non clinical samples. Activities in the laboratory releases biohazards, which may cause damage to the persons involved and associated ecosystem.

To overcome the biohazard related problem in a good laboratory management system should be used.

Containment in microbiology laboratory are divided into two. They are Primary containment and Secondary containment. Primary containment offers protection to personnel and immediate laboratory environment whereas secondary containment offers protection to the environment outside the laboratory.

Primary containment adhering to standard microbiological practices and techniques and awareness of potential hazards. Secondary containment is concerned with proper design of the laboratory facility.

Biological safety cabinets are used depends on the risk group of microorganisms in the laboratory.

Good microbiological techniques should be used to avoid contamination.

Biosafety cabinets provides containment of infectious materials generated during laboratory procedure.

Decontamination, sterilization and disinfection should be done regularly to avoid accidental infections.

Biohazard generated in the laboratory should be segregated and disposed properly.

Collection and transport of Clinical samples

Outcome of microbiology laboratory results completely rely on the type of sample used for the isolation of pathogens.

Sample should be collected from actual infection site, correct volume needed to conduct experiment and ensure the use of proper device.

After sample collection, it is transcribed to the laboratory with necessary informations.

Appropriate methods and special care are considered to transport clinical samples.

Standard operation procedure should be adopted to collect sample from the site from which sample is collected.

Skin – skin swab, biopsy, Pus, Skin scrapins.

Upper respiratory track – Naso pharyngeal aspirate, Throat swab.

Lower Respiratory Track – Sputum, transtracheal aspiration.

Urinary Track – Mid stream, Catheter Urine.

Gastrointestinal system – Stool, rectal swam, Levin tube.

Ear – Swab

Eye – Swab

Blood – Venipuncture

CSF – Lumbar Puncture

Genetal track – Urethral swab, vaginal swab, urethral discharge, Swab from genetal ulcer.

Check your Progress

1.6. Why specimens are needed in microbiology Laboratory
1.7. Mention methods of sample collection from URI.
1.8. Name the best specimen for detecting Tuberculosis.
1.9. Why intubation is performed.
1.10. Menion sample required during UTI.
1.11. What is Lumbar Puncture?
1.12. Mention the method of blood collection.
1.13. Name the method of CSF collection.
1.14. How do you store clinical samples?
1.15. How do you clean skin before venipuncture?
1.16. What is PID.

1.6 UNIT END EXERCISES

Defnitions/ 2 mark answers

Aerosols
Biohazars
Define safety
Define biocontainment
What is sterilization?
What is disinfection?
Define decontamination
What is specimen?
Swab
Pus
Nasopharyngeal aspirations
Otitis media
Style
Dacryoadenitis
Needle aspiration
AFB
Biopsy
Intubation
Lumpar puncture.
Levin tube
Urethritis
Cystitis
PID Gonococcus
Catheter
Suprabubic aspiration
CSF

Short answers

Give the types of laboratory facility design
How do you classify risky pathogenic microbes? Explain..

Describe different biosafety levels.
Give a brief note on biosafety cabinets
Describe personnel biosafety protective equiplments used in laboratory.
What are the emergencies measures taken in microbiology laboratory?
How do you dispose biohazard waste
Write a detailed not on decontamination procedure.
Explain good microbiological techniques
Mention characteristic features of good specimen
What is the information to be filled in specimen container.
Explain method of blood sample collection

Long answers

Give a brief note on collection and transport of sample from skin
Explain method of sample collection from urinary track.
What are the conditions adopted for stool sample collection.
How do you collect CSF? Explain.
What are suprabubic aspirations/ when it is performed?
Describe transport and storage of samples for virus isolation
How do you collect samples from genital system?
Give a detailed accound on biosafety in microbiology laboratory
Write an essay on various samples to be collected from human body.

1.7. CHECK YOUR PROGRESS QUESTIONS

1.1. Aerosols are generated during pipetting, media preparation, centrifugation, vortexing
1.2. Yes, Biohazard generated from microbiology laboratory able to cause acute and chronic infections.
1.3. Yes, Proper safety protocols are needed in containment laboratory otherswise may leads to infection to workers and threat to surrounding ecosystem.
1.4. Sterilization means complete destruction of microbes form the particular object. Yes it is different from disinfection. Disinfection only reduces the number of pathogenic microorganisms.
1.5. BSC is used for safty handly of microorganism. Here microorganisms are inoculated with personal and environmental safety.
1.6. Specimens are the part of body or host which actually represents actual infectious status of the body. It provides nessary information with referencto infections. Without sample diseases are not diagnosed.
1.7. Throat swab, nasopharyngeal sapirations.
1.8. Early morning sputum is best for detecting TB causative agent.
1.9. Intubation is performed to collect samples from hollow structures like stomach. Levin tube is one of the intubation technique used to collect samples gastric juice from stomach.
1.10. Mid stream urine, catheter urine and suprapubic Urine.
1.11. Lumbar Puncture is a method of sample to collect Cerebrospinal Fluid.
1.12. Venipuncture or needle aspiration is the method of blood collection.

1.13. Lumbar Puncture is the method of CSF collection.

1.14. Clinical samples for microbiology laboratory should be processed as early as possible. If there is any deley it should be stored under refrigerator.

1.15. First clean with green soap then with using 1-2% tincture ofIodine. Iodine is removed with 70% ethanol and allowed to dry completely.

1.16. Pelvic Inflammatory disease, which is due to infection of genetal track. Gonorrhea is one of the PID diseases.

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NOTES

UNIT II MICROBIOLOGICAL EXAMINATION

SUMMARY

2.1. INTRODUCTION

Major concern of clinical microbiologist is to isolate and identify microorganisms from clinical samples rapidly. In India, Physicians recommend laboratory analysis if the disease is not cured in first visit of the patient. Samples will be collected as recommended by the physician. All the samples should be processed as per standard good microbiological techniques. Selective cum differential media also used for the complete recovery of the pathogens from clinical samples. The purpose of laboratory examination is to provide physician with information concerning the presence or absence of microbes. To assist the physician in the diagnosis and treatment of infectious diseases. Microbiology data also valuable in monitoring antibiotic therapy. To provide epidemiological information for defining source of infection.

2.2 OBJECTIVES

After reading this unit, readers will be able to

- Understand collection of clinical samples
- Transport of clinical samples
- Important diseases and its pathogens
- Medium used for the isolation of pathogens.
- Processing of clinical samples
- Identification of pathogens.

2.3 MICROBIOLOGICAL EXAMINATION OF URINE

Introduction

Urine is normally a sterile body fluid. However, unless it is collected properly, it can become contaminated with microbiota from the perineum, prostate, urethra or vagina. The presence of bacteria in urine is called bacteriuria. Significant bacteriuria is usually accompanied by pyuria (pus cells in urine). Infection of the bladder is called cystitisand infection of kidney is called pyelonephritis. *Escherichia coli* is a commonest cause of urinary tract infection.

Possible Pathogens - *Staphylococcus saprophyticus, Pseudomonas aeruginosa, Escherichia coli, Proteus sp., Klebsiella sp., Hemolytic Streptococcus, Enterococcus sp.,*

Medium - Blood agar, MacConkey agar, Cetrimide agar, CLED agar, SS agar, KF streptococcus agar, Nutrient agar.

Sample - Mid stream urine, Cathetral urine, Suprapubic aspiration.

Processing of sample

Macroscopy

Note the appearance of the specimen
Colour of the specimen
Nature of the specimen

Physical appearance of the urine

Normally freshly passed urine is clear and pale yellow-to-yellow depending on concentration. When left to stand, cloudiness may develope due to precipitation of urates in acid urine or phosphates and carbonates in alkaline urine. Urates may give the urine a pink-orange color (Table 2.1).

Table 2.1 - Appearance of Urine during Infection

S.NO	COLOR	INFECTION
1	Cloudy	Bacterial
2	Red and cloudy	Bacterial and urinary Schistosomiasis.
3	Brown and cloudy	Black water fever
4	Yellow brown and green brown	Acute viral hepatitis
5.	Yellow orange	Haemolysis and hepatocellular jaundice
6	Milky white	Bancroftian filariasis

Microscopic Examination of urine

Well mixed fresh urine is placed on a slide and cover with a cover glass. Examine the preparation using the 10x and 40x objectives. Perform gram staining using centrifuged urine also. Findings of microscopic examination are in Table 2.2.

Table 2.2 – Microscopy of Urine

Bacteria, White cells, Pus cells, Red cells, Yeast cells, Epithelial cells, Casts, Crystals and Parasites	Casts of urine are Hyaline, Waxy, Cellular and Granular.

Culturing of urine pathogens

Approximate number of bacteria per ml of urine, can be estimated by using calibrated loop technique (0.002ml capacity or 1/500ml or 20x500=10,000). Urine is diluted up to 10^{-5} and perform pour plate technique by using nutrient agar.(normal urine had less than 10^4 bacteria)

For selective isolation, mix urine properly and inoculate a loopful of urine on Blood Agar, MacConkey Agar, CLED Agar, Cetrimide Agar and SS Agar. Incubate all plates under aerobic condition. Table 2.3 illustrates colony morphology of pathogens on selective cum differential media.

Table 2.3 – Colony morphology of Pathogens

Staphylococcus aureus	***Escherichia coli***
Blood agar – β Haemolytic colony Mannitol salt agar – Yellow colour colony Baired parker agar – Black Colour colony Vogel -Johnson agar -Black Colour colonywith yellow background DNase test medium – Pink colour colony	Eosin Methylene Blue Agar – Metallic sheen colonies Xylose Lysine Deoxy Cholate Agar – Yellow colour colonies Salmonella Shigella agar – pink colour colonies Rajhans medium – Greenish colonies Hektoen Enteric agar – Salmon colonies Mac conkey agar- LF colonies
Streptococcus pyogenes, *Blood agar – β haemolytic colony* *Optochin resistant* *Bile non solubilizer*	***Proteus sp.,*** CLED with bromothymol blue – Bluish green colonies XLD agar – Black colonies
Klebsiella pneumoniae *Mac Conkey Agar – Mucoid LF colonies* *Capsulated strain*	***Enterococcus*** Azide dextrose broth – Luxurious growth KF sterptococcus agar with TTC – Pinl minute colonies
	Pseudomonas aeruginosa Cetrimide agar – Greenish blue colonies

2.4. MICROBIOLOGICAL EXAMINATION BLOOD

Introduction

The presence of bacteria in blood is called bacteremia. Transitory bacteremia can occur during the course of many infections. Continuous bacteremia most often suggests an intravascular source of infection. The term septicemia refers to a severe and often fatal infection of the blood in which bacteria multiply and release toxins in the blood stream. The symptoms of septicemia include fever, chills and shock. Bacteria that can be associated with neonatal septicemia include *Escherichia coli,* Staphylococci, Beta hemolytic group B Streptococci and other coliforms.Viridans Streptococcus are the commonest cause of sub acute infective endocarditis. In typhoid, *Salmonella typhi* can be detected in the blood of 75-90% patients during the first 10 days of infection.

Possible Pathogens - Staphylococcus aureus, Viridans Streptococci, Streptococcus pneumonia, Streptococcus pyogenes, Salmonella typhi, Escherichia coli, Klebsiella pneumonia, Corynebacterium diptheriae, Yersinia pestis, Leptospira species, Brucella species, Beta hemolytic group B Streptococci, Proteus species, Haemophillus influenza, Neisseria species.

Medium - Thioglycollate broth, Tryptone soya diphasic broth, Blood agar, Chocolate agar, MacConkey agar, SS agar, EDTA, Materials for WBC and differential count, Giemsa stain

Sample - Blood collection was performed by Needle aspiration procedure. 10-20 ml of blood collected from adult; 1-2.4 ml collected from young infants; 2.4- 5 ml collected from old infants. Two-blood culture should be performed from each patient to confirm the causative agent.

Processing of sample

Microscopyand haematology

Blood is smeared on two slides to perform giemsa and leishman staining.

Total count and differential count is also performed using Haemocytometer.

Haemoglobin and ESR also performed using the sampe blood.

Widal test also performed to check typhod using serum.

Culturing.

Collect 2-5ml of blood from patient. Inoculate two portions into Thioglycollate medium and Tryptone Soya Diphasic Medium. Remaining portion was inoculated into a bottle containing EDTA and used for inoculating SS agar. Incubate all culture bottles and plate at 37°C.

Examination and Sub Culturing

For Thioglycollate broth

- Examine daily for upto 14 days
- Look for visible signs of bacterial growth such as turbidity above the red cell layer.
- A strict aseptic technique must be used to avoid contamination.
- Using a sterile needle and small syringe, insert the needle through rubber liner in the capand withdrawn 1-ml of broth culture.
- Inoculate the broth on Blood agar, Chocolate agar and MacConkey agar
- Incubate blood agar and chocolate agar anaerobically for 48 hours and MacConkey agar plate aerobically for overnight.

For Tryptone soya biphasic culture

- Examine daily for 7 days and twice a week for upto 4weeks
- Look for colonies on the agar slope and signs of bacterial growth on broth.
- If growth is present subculture on blood agar, chocolate agar and MacConkey agar.
- Examine gram stained smear for the colonies.
- If large gram positive rods resembling *C. perfringens* are seen, subculture on Lactose egg yolk milk agar and incubate the plate anaerobically.
- If Brucella is suspected increased attention should be taken and mark as HIGH RISK

All the pure culture isolated from blood are stored properly under refrigerated condition.

2.5 MICROBIOLOGICAL EXAMINATION FAECES

Introduction

Normal human intestine harbors more than 500 types of microbes. Among these some of the microbes are considered as pathogens. Pathogens may enter through food and water systems. Most of the intestinal disorders are based on the toxins.

Possible Pathogens - Escherichia coli, Salmonella enteritis, Shigella sp., Camphylobacter, Vibrio sp., Plesiomonas sp., Aeromonas sp., Yersinia enterocolitica.

Medium MacConkey agar, GN broth, HE agar, TCBS agar, Alkaline peptone water, Caphylobacter isolation medium, Yersinia selective medium

Specimen - Procedure for the recovery of intestinal pathogen is as follows. Collect Stool or rectal swab as specimen. The specimen should be transported as early as possible. Don't refrigerate the stool specimen if possible because certain species of *Shigella species* are susceptible to cooling and drying.

Processing of specimen

Observe the nature and colour of the specimen. Assess Microscopic nature of the specimen by using iodine wet mount technique and gram staining technique. Inoculate the Gram negative broth and alkaline peptone water with few loopful of stool specimen and incubate at 37°C for 4-5 hrs. Observe turbidity. Streak Haektoen Enteric agar by using gram negative broth and TCBS agar with alkaline peptone water inoculum. Observe the plates for the pathogens after 24 hours of incubation at 37°C.At the same time remaining medium given in the medium section are streaked by direct method. After the completion of primary plating techniques, identify the bacterial pathogens using various biochemical tests (Table 2.3).

Table 2.3 Observation - possible growth on Media

Escherichia coli	*Salmonella sp.*
Eosin Methylene Blue Agar – Metallic sheen colonies	Hektoen enteric agar – Dark centerd colony with Green margin
Xylose Lysine Deoxy Cholate Agar – Yellow colour colonies	Xylose Lysine Deoxy cholate agar- Dark centerd colony with Pink margin
Salmonella Shigella agar – pink colour colonies	Salmonella Shigella agar - ark centerd colony with Pink margin
Rajhans medium – Greenish colonies	
Hektoen Enteric agar – Salmon colonies	Bismuth sulphite agar – Black colonies
Mac Conkey agar- LF colonies	Rajhans medium – Reddish colonies
Mac Conkey Sorbitol Agar – Colourless indicates	

Shigella sp. Xylose Lysine Deoxy Cholate Agar – Colourless colonies Salmonella Shigella agar– Colourless colonies Hektoen Enteric agar– Colourless colonies Rajhans medium – Colourless colonies	*Campylobacter sp.,* Campy CVA agar – Colourless colonies Campy Food ID agar – Brown colour colonies
Vibrio cholerae Alkaline peptone water – Luxurious turbid growth TCBS agar – Circular yellow colour colonies	*Plesiomonas sp.,* *Chrome agar – Yellow colour colonies*
Aeromonas sp., *Chrome agar – Brown colour colonies* Inositol brillient green bile agar – Colourless colonies Blood agar with 10 µl/mL ampicillin – Pale coloured colonies CIN agar – Circular pink colonies	*Yersinia enterocolitica* CIN agar – Pink small colonies XLD agar – Yellow colonies Blood agar – Yellowish brown colonies PSTA enrichment broth – Luxurious growth

2.6 MICROBIOLOGICAL EXAMINATION CEREBROSPINAL FLUID

Introduction

Bacterial meningitis is the result of infection of the meninges. Identification of the infecting agent is one of the most important functions of the diagnostic laboratory because acute meningitis is a most serious infection. Aerobic bacteria commonly cause meningitis. Inoculation of anaerobic bacteria is not recommended. CSF may contain very few microorganisms per ml of fluid, therefore, concentration of the specimen is recommended. Any positive finding on gram stain or culture must be reported to the physician immediately.

Possible Pathogens - Escherichia coli, Streptococcus agalactiae, Listeria monocytogens, Haemophillus influenza, Neisseria meningitis and Streptococcus pneumoniae.

Specimen - CSF. It is collected by Lumbar puncture and Brain abscess collection method. The chance of recovery increases with the volume of the specimen. Suggested volumes are 1ml for bacterial culture and 2ml for fungus . Don't refrigerate the specimen.

Media - Blood agar, Chocolate agar, Modified Thayer martin agar, New York city agar medium and G.C Medium

Processing of CSF

Macroscopy

Record gross appearance of CSF. Centrifuge specimen. Aspirate the supernatant. Vortex the sediment vigorously for at least 30 seconds.

Microscopy

Prepare the smear by placing one or two drops of sediment on an alcohol rinsed slide allow in the drop to form a large heap. Fix the smear by using methanol and perform gram staining. Examine the slide and report physician immediately if there are any positive findings.

Culturing

Using sterile pipette inoculates media by placing one or two drops of the vortexed sediment on two plates of Blood agar and chocolate agar. Incubate all plates at 35°C in 5 to 10 % carbondioxide containing environment for 48 to 72 hrs. Examine all plates for growth. If growth is observed, inoculate isolates on selective cum differential media like Modified Thayer martin agar, New York city agar medium and G.C Medium. Colony morphology on selective medium confirs the identity of the organism or perform biochemical test to complete the organism identity.

2.7 MICROBIOLOGICAL EXAMINATION THROAT SWABS

Introduction

A throat swab culture, or throat culture, is a test commonly used to diagnose bacterial infections in the throat. These infections can include strep throat, pneumonia, tonsillitis, whooping coughand meningitis.

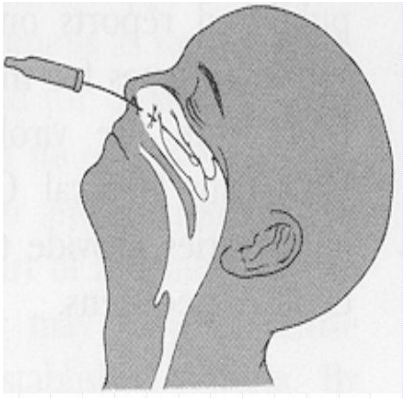

Fig. 2.1 – Nasopharyngeal swab

The purpose of a throat swab culture is to detect the presence of organisms in the throat that could cause infection. For example, the presence of group A streptococcus bacteria (Streptococcus pyogenes) in your throat is a key sign that you may have strep throat. Infections affecting the throat (larynx) or the main airway (trachea) or the airways going into the lungs (bronchi) are common. These infections are sometimes called laryngitis, trachitis or bronchitis. Doctors often just use the term URTI (upper respiratory tract infection) to include any or all of these. Cough is usually the main symptom. Other symptoms include fever, headache, aches and pains. Cold symptoms may occur if the infection also affects the nose. Symptoms typically peak after 2-3 days and then gradually clear. However, the cough may persist after the infection has gone. This is because the inflammation in the airways caused by the infection can take a while to clear. It may take up to 4 weeks after other symptoms have gone for the cough to clear completely.

Possible Pathogens - Streptococcus pyogenes, Other beta haemolytic Streptococci, Bordetella pertusis, Streptococcus pneumoniae, Haemophillus influenzae, Corynebacterium diphtheriae, Pseudomonas aeruginosa, Borrelia sp., Bacteroides malaninogenicus, Neisseria meningitis, Klebsiella sp., Staphylococcus aureus

Specimen - Sodium alginate throat swab for Pertusis, Sinus washings, Surgical biopsy, Swab of posterior pharynx, Swab of tonsil, Pernasal swab, Nasopharyngeal aspirates- Gently pass a sterile catheter through one tonsil as far as the nasopharynx. Attach a sterile syringe to the catheter and aspirate the specimen of mucopus. Dispense the specimen into a sterile container.

Nasopharyngeal swab - Insert a **sterile dry swab** into the nasal cavity of the patient and wipe the swab along the sides of the nasal passage. Place the swab with the cotton tip end into the small vial (or leakproof screw top tube with 3-5 ml volume) of virus transport medium.

Throat swab -Bright light from over the shoulder of the patient should be focused in to the oral cavity. The patient is instructed to open the mouth at 'aah' position and breathe deeply. The tongue is gently depressed with tongue blade to visualize the tonsillar fossae and posterior pharynx. The swab is extended between tonsillar pillars and behind the vulva. The tonsillar areas and posterior pharynx should be firmly rubbed with the swab. After collection the cotton swabs are placed into sterile Cary Blair transport media to prevent desiccation during transit to the laboratory.

*Media***:** Blood agar plate, Cystine tellurite blood agar, Loeffler agar slant, Chocolate agar, Charcoal Cephalexin Blood Agar (CCBA), Modified tindsale medium, Saboured dextrose agar, Thayer martin agar, Cetrimide agar, New York City Agar Medium, Baired Parker Agar, Mac Conkey agar. Alberts staining reagent, bacitracin disc etc.

Procedure

Microscopy

Alberts Staining Technique

Culturing

Sample of swab is inoculated on a plate of blood agar. Bacitracin disc is placed on the plate. This will help in the identification of *Streptococcus pyogenes*. Use tinsdale's medium and Tellurite blood agar for the recovery of *Corynebacterium diphtheria*. Use chocolate agar for the isolation of *H.influenzae, N. meningitides*. Use charcoal cephalexin blood agar and Bordet Gengou medium for the recovery of *Bordetella pertusis*.

Use MacConkey agar to isolate Gram Negative pathogens. After inoculation all the plates are incubated at appropriate temperature for a required time. Colny morphology is observed and confirm the bacterial identity.

Table 2.4 -Observation - possible growth on Media

Staphylococcus aureus Blood agar – β Haemolytic colony Mannitol salt agar – Yellow colour colony Baired parker agar – Black Colour colony Vogel -Johnson agar -Black Colour colonywith yellow background DNase test medium – Pink colour colony	***Streptococcus pneumoniae*** *Blood agar – α haemolytic colony* *Capsulated strains produce α haemolytic mucoid colony.* *Optochin sensitive* *Bile solubilizer*
Streptococcus pyogenes, *Blood agar – β haemolytic colony* *Optochin resistant* *Bile non solubilizer*	***Corynebacterium diphtheriae*** Loeffler agar – Luxurious growth Cysteine tellurite blood agar-Black colour colony Blood agar- β haemolytic colonies
Klebsiella pneumoniae *Mac Conkey Agar – Mucoid LF colonies* *Capsulated strain*	***Haemophillus influenzae*** Levinthols medium- colourless colonies Chocholate agar- grey coloured colonies Satellism
Neisseria meningitides New York City agar medium-Small ash coloured colonies Modified Thayer martin Agar-Small colourless colonies	***Pseudomonas aeruginosa*** Cetrimide agar – Greenish blue colonies
	Bordetella pertusis Bordet –Gengou medium – Glistening mercury like colonies Rogen-Lowey medium – Ash coloured colonies

2.8 MICROBIOLOGICAL EXAMINATION SPUTUM

Introduction

Lower respiratory tract (LRT) infections involve lung and bronchi. Normally Lower respiratory tract should be sterile. Any organism that is able to by-pass the host defenses, enter the Lower respiratory tractand multiply is capable of causing diseases in LRT. Sputum is collected during lung infections especially during pneumonia and tuberculosis.

Possible Pathogens - Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Mycobacterium tuberculosis, Klebsiella pneumoniae, Beta haemolytic group B Streptococci, Haemophillus influenzae, Neisseria species, Pseudomonas aeuginosa, Mycoplasma pneumoniae etc.,

Media **:** Blood agar, Chocolate agar, Mac Conkey agar, Baired parker agar, Cetrimide agar, LJ medium, Buffered charcoal yeast extract agar, Modified Thayer martin agar, New york city agar medium.

Specimen - Collect Early morning sputum. It contains pooled overnight secretions with concentrated bacteria. A sterile wide mouth jar with a tight screw cap lid can be used to collect sample..

Transporting - Sputum is transported or stored by adding cetyl pyridinium chloride - sodium chloride (CPC-NaCl) It digests sputum and prevents the over growth of other pathogens.

Processing
Procedure
Microscopy

The gram stain can aid in rapid diagnosis and appropriate treatment. The following cells were observed after staining, they are PMN, squamous epithelial cells, ciliated columnar epithelial cells and bacteria. Acid Fast Staining is performed to detect tubercle bacilli.

Culturing
Routine

Wash the purulent part of the sputum with sterile 5mL of physiological saline. Inoculate washed sputum on plates of blood agar and chocolate agar. Add optochin disc to the chocolate agar. This will help to identify *Streptococcus pneumoniae.* Incubate the blood agar plate aerobically and chocolate agar plate in a carbon di oxide enriched atmosphere at 35-37 ° C for upto 48 hours. Examine the growth and report the result. For AFB (Acid Fast Bacilli – *Mycobacterium tuberculosis*)

About 20 minutes before culturing decontaminate the specimen by mixing equal volumes of sputum and NaOH 40g/l solution. Shake at intervals to homogenize the sputum. Using a sterile premarked Pasteur pipette, inoculate 200µL of the sputum on a slope of acid Lowenstein Jensen medium. Allow the specimen to run down the slope. Slope turn yellow due to alkalinity of the specimen but it will become green again acid in the medium neutralizes the NaOH. Incubate the tubes at 37°C in rack placed at an angle of about 45° to ensure that the specimen is in contact with the full length of the slope. After one week, place the slopes in an upright position and continue to incubate the cultures for a further 5-6 weeks, examine twice a week for growth.
For other pathogens

Streak the sputum on selective and differential medium for the isolation and differentiation, as per clinical diagnosis and recommended by the physician.

2.9 MICROBIOLOGICAL EXAMINATION PUS AND WOUND EXUDATES

Introduction

Wound is an abnormal break in the skin or other tissue, which allows blood to escape. Wounds are two types, they are open wound and closed wound. Open wound allows blood to escape from the body. Here the skin is broken. All open wounds are contaminated by germs, which enter from air, fingers and other parts of the body. Any wound, which has not begun to heal properly after 48 hours it, may be infected. Infection may further spread and cause dangerous illness to human beings.
Possible pathogens - *Staphylococcus aureus* mostly isolated from skin wounds, *Pseudomonas aeruginosa* is associated with infected burns and with hospital acquired infections. *Escherichia coli*, *Proteus species* associated with abdominal abscess. *Clostridium perfringens* is found mainly in deep wounds. Wound infections may be caused by one to many organisms depending on the site of the infection. Postoperative wound are often infected with a mixture of aerobes and anaerobes, while deep wound infections such as internal body or organ infections can be caused by one or several aerobes and/or anaerobes.

Specimen Collection - As far as possible, collect specimens before antimicrobial therapy and/or before application of antiseptic dressing. The ideal specimen is an aspirate from a previously undrained abscess, or a tissue biopsy. Ideally, a minimum volume of 1mL (up to 5 mL) of pus should be collected. Large volumes of purulent material maintain the viability of anaerobes for longer.

The aspirate should be collected in a sterile syringe – any air bubbles should be expelled. Needle safely and tightly capped. A tissue specimen should be placed in a sterile universal bottle (or any sterile leak proof container) and sent to the lab for immediate processing if anaerobes are suspected. If there will be a delay in transporting, the tissue should be placed in an anaerobic transport system. Swabs are less desirable because of the smaller amount of specimen that is sampled and the fact that they are often contaminated with normal skin flora, making interpretation of results difficult. When using swabs, the deepest part of the wound should be sampled, avoiding the superficial microflora. Swabs should be well soaked in pus.

Specimen Transport: Label the specimen and deliver it to the laboratory as soon as possible with a completed request form. The volume of specimen and the nature of the suspected organism influences the acceptable transport time. The recovery of anaerobes is compromised if the transport time exceeds 3hr. If delays in transportation to the laboratory are unavoidable sample should be placed in the transport medium (Amies transport medium or **Cary-Blair transport medium**) to minimize drying and minimize exposure to oxygen if anaerobes are suspected.
Processing of Sample
Gram Staining: Make an evenly spread smear of the specimen on a clean, grease-free slide. Allow the smear to air-dry in a safe place. Heat fix the specimen and stain by gram staining technique. Examine the smear for the presence of bacteria and pus cells (PMNs) using 100x objective lens and look especially for: Gram negative rods

(*E.coli*, ***Klebsiella pneumoniae***, ***Pseudomonas aeruginosa***, *Proteus* or Bacteroides species), Gram positive cocci in pairs, chains or clusters (***Staphylococcus aureus***, ***Streptococcus pyogenes,*** anaerobic streptococci or enterococci). Gram positive large rods with square ends (*Clostridium perfringens*or *Bacillus anthracis*). Sometimes, Gram positive yeast cells with psuedohyphae may be seen, which can be ***Candida albicans***.

Pus Culture

Inoculate pus sample on selective cum differential medic and observed following. In the Blood Agar plate, both Staphylococus aureus and Streptococcus pyogenes gives beta-hemolysis . S. aureus gives yellow to cream or white colonies. Colonies are slightly raised and easily emulsified. S.pyogenes produces beta-hemolytic colonies. Colonies are usually small, colourless, dry, shiny or mucoid. Enterococci gives non-hemolytic colonies in blood agar. We can differentiate between streptococci and staphylococci by a very simple and rapid test-Catalase test (Staphylococcus-positive, streptococcus-negative). For identification of suspected S. aureus colonies perform coagulase test (to differentiate Coagulase negative Staphylococci from S. aureus) and for suspected Group A Streptococci (S.pyogenes) preform bacitracin sensitivity test (can be added in the blood agar plate with other antibiotics). If enterococci is suspected perform bile esculin test.

Lactose fermenting colonies on Mac Conkey agar can be of *Escherichia coli*, Klebsiella *sps or* Enterobacter *sps* and non-lactose fermenter colonies can be of Psuedomonas aeruginosa, Acinetobacterspp, Proteus spps etc. Member of the family of the Enterobacteriaceae can be differentiated from other Gram negative bacilli by performing two rapid tests (catalase test +veand oxidase test –ve). Identifications of the enteric bacteria can be done by using biochemical tests such as Citrate utilization test, Triple Sugar Iron (TSI) Agar test, Sulphite-Indole–Motility (SIM) testand Urease test. Pseudomonas aeruginosa gives large, flat, spreading pale colored colonies in MacConkey Agar. It is oxidase positive and can be identified by its pigments and/or distinctive smell (characteristics fruity smell).

Check Your Progress

2.1. Why human blood is not used for the preparation of blood agar.

2.2. What is biphasic medium?

2.3. Why *E.coli* is the commonest cause of UTI.

2.4. How do you collect samples to detect UTI?

2.5. Name the commonest medium used for the recovery of G-pathogens.

2.6. Is there any normal flora in throat?

2.7. Name the medium used for the cultivation of *C.diphtheriae.*

2.8. When did you collect sputum?

2.9. Why there is a need for early morning sputum.

2.10. Name the medium used for the recovery or tubercle bacilli.

2.11. How pathogens enters in to human intestinal system.

2.12. Is microscopy necessary for wound pathogen isolation?

2.10. LET US SUM UP

Urine

Microbiologists are involved in processing of clinical samples to diagnose diseases and its causative agents. Microbiologis helps physican to identify the diseses, its agents and the medicine required for the correct treatment.

Sample is collected in microbiology laboratory as per the instruction of physician and standard Operation Procedure. Mid stream / Catheter/ Suprapubic Urine is collected during UTI infections like cystitis, pyelonephritis etc.and transported to the laboratory as early as possible.

Samples are processed macroscopically, microscopically and culture using selective cum differential medium. On the basis of colony morphology and biochemical pattern the etiological agents are identified.

Blood

Blood samples are collected in septiceamic condition. Bacteria associated with blood are *Escherichia coli*, *Staphylococci*, Beta hemolytic group B *Streptococci* and other coliforms.Viridans Streptococcus are the commonest cause of sub acute infective endocarditis. In typhoid, *Salmonella typhi.*

Blood sample is collected by venipuncture method/ Needle aspiration method. Blood sample is inoculated on Thioglycollate medium, tryptone soya biphasic medium. Both medium are incubated for 7-14 days and observed for growth.

Culture from Thioglycollate medium, tryptone soya biphasic medium are subcultured on selective cum differential medium like Blood agar, Mac Conkey agar etc.,

Blood sample also subjected for DC, TC, HB, ESR analysis, Giemsa, Leishman staining.

Growth on selective cum differential media and biochemical characterizations confirms the identitiy of blood borne pathogens.

Stool

Diarrhoeal stools samples are collected for the analysis of enteric pathogens like bacteria, protozoa and helminthes.

Sample is processed microscopically and culture of selective cum differential media. Saline and iodine wet mount are performed to detect protozoans and helmonthes. Stool is incoculated on TCBS agar to detect Vibrio sp., Haekotein enteric agar for the detection of other enteric pathogens. Mac Conkey agar also inoculated.

Organisms identity is confirmed by making used of cultural and biochemical characters.

Cerebrospinal Fluid.

Bacterial meningitis is the result of infection of the meninges. Identification of the infecting agent is one of the most important functions of the diagnostic laboratory. CSF may contain very few microorganisms per ml of fluid, therefore, concentration of the specimen is recommended. CSF is collected by Lumbar puncture and Brain abscess collection method. Gram staining is performed detect any bacterial agents. Culturing is done by placing one or two drops of the vortexed sediment on two plates of Blood agar and chocolate agar. Incubate all plates at 35°C in 5 to 10 % carbon dioxide containing environment for 48 to 72 hrs. If growth is observed, inoculate isolates on selective cum differential media like Modified Thayer martin agar, New York city agar medium and G.C Medium. Colony morphology on selective medium confirms the identity of the organism or perform biochemical test to complete the organism identity.

Throat Swabs

A throat culture is used to diagnose bacterial infections in the throat. These infections can include strep throat, pneumonia, tonsillitis, whooping coughand meningitis. The purpose of a throat swab culture is to detect the presence of organisms in the throat that could cause infection.

Streptococcus pyogenes, Other beta haemolytic Streptococci, Bordetella pertusis, Streptococcus pneumoniae, Haemophillus influenzae, Corynebacterium diphtheriae, Pseudomonas aeruginosa, Borrelia sp., Bacteroides malaninogenicus, Neisseria meningitis, Klebsiella sp., Staphylococcus aureus are major pathogens of URT. Sample of swab is inoculated on a plate of blood agar. Bacitracin disc is placed on the plate. This will help in the identification of *Streptococcus pyogenes*. Use tinsdale's medium and Tellurite blood agar for the recovery of *Corynebacterium diphtheria*. Use chocolate agar for the isolation of *H.influenzae, N.meningitides*. Use charcoal cephalexin blood agar and Bordet Gengou medium for the recovery of *Bordetella pertusis*. Use MacConkey agar to isolate Gram Negative pathogens. After inoculation all the plates are incubated at appropriate temperature for a required time. Colny morphology is observed and confirm the bacterial identity.

Sputum

Lower respiratory tract (LRT) infections involve lung and bronchi. Sputum is collected during lung infections especially during pneumonia and tuberculosis. The gram stain can aid in rapid diagnosis and appropriate treatment. The following cells were observed after staining, they are PMN, squamous epithelial cells, ciliated columnar epithelial cells and bacteria. Acid Fast Staining is performed to detect tubercle bacilli. Wash the purulent part of the sputum with sterile 5mL of physiological saline. Inoculate washed sputum on plates of blood agar

and chocolate agar. Incubate the blood agar plate aerobically and chocolate agar plate in a carbon di oxide enriched atmosphere at 35-37 ° C for upto 48 hours. Examine the growth and report the result. For AFB (Acid Fast Bacilli – *Mycobacterium tuberculosis*) - Using a sterile premarked Pasteur pipette, inoculate 200µL of the homogenised sputum on a slope of acid Lowenstein Jensen medium. Allow the specimen to run down the slope. Incubate the tubes at 37°C. After one week, place the slopes in an upright position and continue to incubate the cultures for a further 5-6 weeks, examine twice a week for growth. Streak the sputum on selective and differential medium for the isolation and differentiation, as per clinical diagnosis and recommended by the physician.

Pus and Wound Exudates

Wound is an abnormal break in the skin or other tissue, which allows blood to escape. *Staphylococcus aureus* mostly isolated from skin wounds, *Pseudomonas aeruginosa* is associated with infected burns and with hospital acquired infections. *Escherichia coli*, *Proteus species* associated with abdominal abscess. *Clostridium perfringens* is found mainly in deep wounds. Inoculate pus sample on selective cum differential medic and observed following. In the Blood Agar plate, both Staphylococus aureus and Streptococcus pyogenes gives beta-hemolysis. S. aureus gives yellow to cream or white colonies. Colonies are slightly raised and easily emulsified. S.pyogenes produces beta-hemolytic colonies. Colonies are usually small, colourless, dry, shiny or mucoid. Enterococci gives non-hemolytic colonies in blood agar. Lactose fermenting colonies on Mac Conkey agar can be of *Escherichia coli*, Klebsiella *sps or* Enterobacter *sp*s and non-lactose fermenter colonies can be of Psuedomonas aeruginosa, Acinetobacterspp, Proteus spps etc.

2.11. UNIT END EXERCISES

Why human blood is not used for the preparation of blood agar.
List out blood borne infection of human.
How do you cultivate typhoid-causing pathogens from blood?
Define Bacteremia, transitory bacteremia, septicemia and toxemia.
Define bacteriuria, pyuria, cystitis, pylonephritis.
What are possible pathogens of wound?
Define mid stream urine, cathedral& suprabubic aspiration.
How colour of specimen gives picture of possible TI.
What are the microscopic structures are visualized, during suspected UTI.
Why CLED medium is used to recover major UTI pathogens.
Name the commonest medium used for the recovery of G-pathogens.
Name the medium used for the recovery of Proteus.
Which organism is identified using TCBS agar.
What are the possible pathogens of throat?
Is there any normal flora in throat?
Mention few viral and bacterial infection of throat.
Name the medium used for the cultivation of *C.diphtheriae.*
How do you differentiate *S. aureus* & *C. diphtheriae*
Name the medium used for the recovery or tubercle bacilli.
What are the possible bacterial pathogens of LRT?

How do you grade sputum sample.
Name the medium used for the cultivation of haemolytic Streptococuus.
How do you differentiate Staphylococcus & Streptococcus?
Define AFB.
What are all the ingredients of LJ medium?
List bacterial pathogens associated with gastro intestinal system.
List some of the selective medium used for the differentiation EHEC from other *E.coli*.
Name the medium used for the differentiation EHEC from other E.coli.
How do you confirm pathogenic *E. coli*?
How do you differentiate types of *E. coli*?
Name the medium used for the cultivation of vibrio
What are the uses of blood agar?
Is microscopy necessary for wound pathogen isolation?
What are the advantages of microscopy in pathogen identification from clinical samples?
What kind of specimen is used for the recovery of wound pathogen?
What are the principles of Mac Conkey agar & blood agar?
Define bacteriuria, pyuria, cystitis, pylonephritis.
Describe isolation bacteria from stool.
Explain procedure to isolate bacteria from urine.

2.12. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

2.1. Human blood contain antibodies against the pathogen to be isolated, which interfere or inhibit the growth of pathogens.
2.2. Biphasic medium means both solid and liquid portions are available in a singe tube/ bottle.
2.3. This Bacterium is available as normal flora in intesting and it easily get accessible to UT from anus and predominatly caus UTI
2.4. Mid stream, Catheter and Subrapubic Urine.
2.5. MacConkey agar.
2.6. Yes, multiple numbers are there as normal flora eg. Streptococcus
2.7. Loeffler serum Slope
2.8. Early morning
2.9. Early morning sputum pooled microbial population, which increases the chance of recovery..
2.10. LJ Medium
2.11. Pathogens enters in to human intestinal system through food and water.
2.12. Yes, microscopic analysis provide initial pictue of pathogens, media to be selected and type of treatment needed.

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UNIT III NORMAL FLORA OF HUMAN SYSTEMS

Structure

3.1 INTRODUCTION

There are countless microorganisms in the environment, within this environment, human beings encounter these microbes continuously. But our intimate contact is with the large number of microbes that actually live in and on our bodies. It is estimated that the adult human body is host to at least 100 trillion microbial cells at any time. These indigenous microbes most of which are bacteria are called the normal flora. The human body is inhabited by large number of microbes, which together are called body's Normal Flora.

3.2 OBJECTIVES

Study of this unit will be able to

- Differentiate pathogen from normal flora.
- Understand benefits of normal flora.
- Understand nonspecific defence mechanism of human body.
- Understand the concept of nosocomial infection.
- Study the role of animate and inanimate factors in the spread of disease.
- Control of nosocomial infection
- Committee associated with the control of hospital borne infections.

3.3. GENERAL FEATURES OF NORMAL FLORA

Normal flora inhabits the skin and some of the inner parts of the body. The species and number of the flora vary according to the particular sites and particular age of the host. Sex of the host may also influence the nature of normal flora. Normal flora benefits from the host but the host is not adversely affected. Many sites of human body are free from microbes. These include the cerebrospinal fluid, blood, urinary bladder, uterus, fallopian tubes, middle ear, Para nasal sinuses and kidneys. Normal flora is also called residents of the healthy human host. Normal floras are permanent occupants. Transients may establish brief contact with human body, but are excluded by competition residents or by host immune system. Some normal flora becomes a pathogen when host defense faulter or any damage in human body. Normal Flora rise over all immune status of the body against pathogens having related or shared antigens.

Nature of normal flora depends on the environment, hygienic practices and frequency of washing. There are four reasons to acquire knowledge of the normal flora of the human body. To study interactions between resident flora and transient flora. Help physician to evaluate the nature of infection. To know what kind of microbes present in and on our body. Increased awareness about normal flora.

3.4. ORIGIN OF THE NORMAL FLORA

Before birth a healthy human fetus is free of microbes. With in hours after birth, it begins to acquire a normal micro biota, which stabilizes during the first week or second week of life. From then on, enormous numbers of microorganisms associates with human body.

3.5. NORMAL FLORA OF HUMAN SKIN

It is one of the most understandable regions in the body. It provides good barrier system and is considered as a first line defence system in the body. It has epidermis, dermis, subcutaneous connective tissue, sweat glands, sebaceous glands and hair follicles etc. The skin varies widely in structure and function, depending on its location on the body. These differences determine the types and numbers of microbes that occur on each skin site. Commensal microbes living on or in the skin can be either resident or transient microbiota. Resident microbiota normally grow on or in the skin. Constant exposure to the environment means the skin harbors many transient microbes. Transient microbes are unable to multiply and normally die in a few hours.

Several factors are responsible for discouraging microbial growth on the skin.

Dryness - The relatively dry surface of the skin is inhibitory to microbial growth. When skin is allowed to dry, many bacteria enter dormant condition, some species die within an hour. Moist region of the skin have high number of normal flora.

Low pH - Skin normal pH is about 3 – 5. Some organisms produce organic acids that reduces pH. This low pH can inhibit the growth of many kinds of microorganisms.

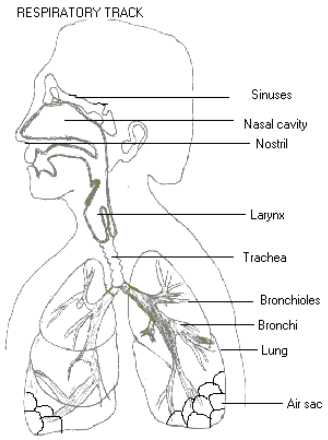
Inhibitory substances- Sweat gland contains high concentration of sodium chloride. This makes the skin surface to osmotically stressed environment. Sweat gland secretes lysozyme, which lyses gram-positive bacterium. Sebaceous gland secrete complex lipid that may partially degraded by the enzymes of gram positive bacteria and forms unsaturated fatty acids such as oleic acids [it has strong antimicrobial activity]. Some of the fatty acids are volatile in nature. It contributes body odour. Instead of these positive responses from the skin some negative responses are also there in skin. Excretions from Sebaceous and sweat gland provide water, aminoacids, urea, electrolytes and fatty acids that serve as nutrients primarily for S*taphylococcus* and C*orynebacterium*. The most prevalent bacterium in skin glands is the gram-positive anaerobic lipophilic rod *Propionibacterium acne*. This bacterium usually harmless. Due to the over secretion of Sebaceous and sweat gland, *P.acne* growth is enhanced. This leads to the formation of the disease acne vulgaris. Accumulation of large amount of sebum provides microenvironment for the growth of normal flora. Accumulation of large amount of sebum also leads to inflammatory response that causes redness and swelling of the gland duct produces *comedo* (a plug of sebum and keratin in the duct). Inflammatory lesions commonly called "black heads or pimples"

Coagulase negative – Staphylococcus
Staphylococcus aureus
Propionibacterium acnes
Bacillus spp.
Streptococci
Malassezia furfur
Candida spp.
Pityrosporum

3.6 NORMAL FLORA OF HUMAN RESPIRATORY TRACT

The respiratory tract includes nose, tonsils, nasopharynx, throat, trachea, bronchi and lungs. The upper respiratory tract consists of nose, tonsils, nasopharynx and throat. The mucous membrane of the upper respiratory tract is moister than skin, nevertheless, they can create more problems to microorganisms.

Staphylococcus aureus and Staphylococcus epidermidis is the predominant flora of nose and sometime it has some microflora as like that of skin of face. Nasopharynx that part of pharynx may contain small number of S*treptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenza*. Most of these organisms lack capsule. *Diptheroids,* a large group of gram positive organism presents in nose and nasopharynx.



Anatomy of Human Respiratory Tract

Oropharynx is the division of

pharynx, most important bacteria found in this area are the various alpha haemolytic S*treptococci* (*S.oralis, S.milleri, S.gordoni, S.salivaris*) large number of *Diptheroids, Branhamella,* small gram negative cocci,. Tonsil harbours a similar microbiota of oropharynx but within the tonsillar crypts, there is an increase in M*icrococcus* and anaerobes (*Porphyromonas and Fusobacterium).*

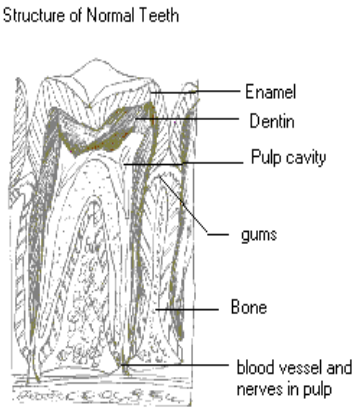
Because of the rhythmic beating of cilia on the nasopharynx, mucous membranes of nasopharynx continously flow organisms towards the Oropharynx. The trapped bacteria are eventually swallowed and may be destroyed by Hcl of stomach. In addition this mechanical removal of bacteria, the enzyme lysozyme in nasal mucous kill bacteria

Normally lower respiratory tract do not have any normal microbiota, because of the efficient mechanical removal by mucous and cillia, most of the bacteria usually are engulfed and destroyed by phagocytic action of alveolar macrophages.

3.5 NORMAL FLORA OF HUMAN GASTROINTESTINAL TRACT

Normal microbiota of the oral cavity contains those organisms that are able to resist mechanical removal by adhering to surfaces like gums and teeth. Oral cavity serves as a ideal environment for microbes because it provide moistureand food material. However continuous desquamotion of epithelial cell, flow of saliva and mechanical flushing action removes microbes from the oral cavity.

At birth the oral cavity is essentially a sterile, warm and moist cavity containing variety of nutritional substances. The saliva is composed of water, aminoacids, proteins, lipids, carbohytrates and inorganic compounds. The newborne establish its normal flora within few days after birth. The oral cavity is colonised by microorganisms from external environment. Initially, the microbiota consist mostly of the genera *Streptococcus, Lactobacillus, Neisseria, Actinomycetes*. Some yeast is also present. The number and kinds of microbes present in mouth depends on the diet and to association with other peoples, objects such as towelsand feeding bottles. The only species isolated from the mouth through out the life is *S.salivaris.* It has an affinity to epithelial tissues and appears in large number on upper surface of the tongue.



Until eruption of teeth most microbes present in the teeth are aerobes and facultative anaerobes. As the first teeth erupt the anaerobes become dominant due to the anaerobic nature of the gingival groove. As the first tooth grows *Streptococcus gordonii* and

S.mutans attach to their enamel surface, *S.salivaris* attaches to the bucal and gingival epithelial surfaces and colonises the saliva. These Streptococci produce various adherence factors. These factors enhance the attachment of oral bacteria. The aggregation of bacteria on organic matter of the teeth surface is termed dental plaque. These organisms allow the secondary invaders and forms dental caries, gingivitis and periodental diseases. Periodental disease is caused by several group of bacteria.

Stomach receives different transient organisms from the oral cavity. The stomach usually contains less than 10 viable bacteria per milliliter of gastric juice. This is due to the bactericidal effect of gastric hydrochloric acid and digestive enzymes. Organisms present in stomach include *Staphylococci, Streptococci, Lactobacillus, Peptostreptococcus* and yeast. Microorganism may survive if they pass rapidly through the stomach or if the organism ingested with food are particularly resistant to gastric pH. Changes in gastric microbiota also occur if these is any increase in gastric pH, this leads to stomach upset. If pH is increase stomach is filled with both gram negative anaerobic and aerobic bacteria. Following the ingestion of food the number of microbes' increases then gradually decreases.

The small intestine is divided into three areas. They are Duodenum, Jejunumand illeum. Duodenum contains small number of microrganisms because combined action of strongly acidic environment of the stomach and the inhibitory action of bile and pancreatic secretions of the bacteria present, gram-positive cocci and rod comprise most of the microbiota. *Enterococcus faecalis. Lactobacilli*, *Diptheriods* and *Candida albicans* are occasionally found in the jejunum. In illeum the microbiota begins to take on the characteristics of the colen micrbiota .It is within the illeum that the pH becomes more alkaline. As a result anaerobic gram negative bacteria and the members of the family *Enterobacteriaceae* become established.

Colon has the largest microbial population in the body. It has been estimated that the number of microorganisms in stool specimen is 10^{12} organisms per gram wet weight, which means that about 25% of feces is made up of microorganisms. Over 300 different bacterial species have been isolated from human feces. It has been estimated that an adult excretes 30 million bacterial cells daily through defecation.

The colon is considered as a large fermentation vessel because it contains anaerobic, gram negative nonsporing bacteria and gram positive sporeforming and non sporeforming bacteria and yeast, protozoa may occur as harmless commensals, *Trichomonas hominis*, *Entamoeba hartmanni, Endolimax nana* and *Entamoeba butschli* are common in-habitats. These organisms do various functions like fermentation.

Various factors tend to remove microorganisms from the large intestine. One factor is the peristalic movement of the intestinal contents. Desquamation of surface epithelial cells to which bacteria are attached to

another factor. Third factor is the mucous, which removes microbes by mechanical process as like respiratory tract.

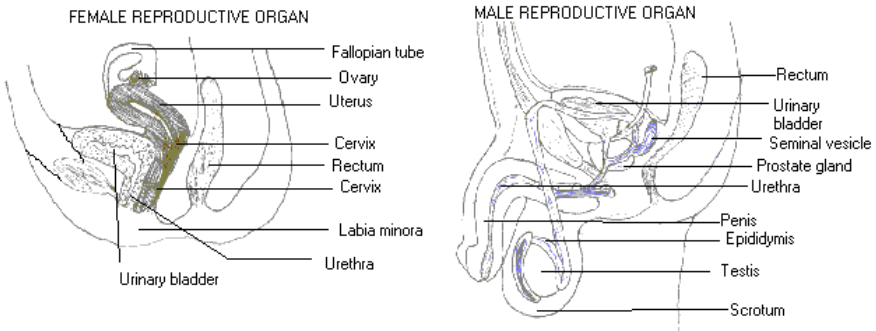
The initial residents of the colon of breast-fed infants are members of the gram positive Bifidobacterium because human milk contains a disaccharide aminosugar which is required for the growth of Bifidobacterium. In formula fed infants, Lactobacillus species are predominate. Composition of the normal flora of the intestine can be influenzed by various factors such as strong emotinal stress and starvation.

Intestinal bacteria contribute to intestinal odour by producing skatole, amines and gases (CO_2, H, CH_2 and H_2S). The bacteria produce an average of 8.5liters of gas daily.

3.6 NORMAL FLORA OF HUMAN GENITOURINARY TRACT.

The upper genitourinary tract (kidney, ureters and urinary bladder) is usually free of microbiota. The regions of the genitourinary tract that harbour microflora are the vagina and outer opening of the urethra in female and anterior opening in the males. The internal reproductive organs are kept sterile through physical barriers such as the cervical plug and other host defences. The kidney, ureter, bladder and upper urethra are kept sterile by urine flow and regular bladder emptying. Since the urethra in women is so short (about 3.5 cm long). It can form a passage for bacteria to the bladder and lead to ferquent UTI

Adult female genital tract pH is about 4.4—4.6. This due to the action of the hormone estrogen. It stimutates the vaginal mucosa to secrete the glycogen, which is metabolised by *Lactobacillus acidophills*, often called doderlein's bacilli and forming lactic acid. The normal flora and others change the mentural cycle during some period.



Normal microbiota of the urethra are coagulase negative *Staphylococci, Diptheroids, Strerptococci, Mycobacterium, Bacteriods, Fusobacterium,, Peptostreptococcus spp*. etc. Normal microbiota of the vagina *are Lactobacillus, Diptheroids, Peptostreptococcus, Streptococcus, Clostridium, Candidaand Gardinerella vaginalis.*

NOTES

Check your progress

3.1. Why is there a huge number of normal flora in the colon

3.2. State the reason behind lungs being free from microorganisms

3.7. NOSOCOMIAL INFECTIONS.

Introduction

Nosocomial infections are infections acquired in the hospital. The term nosocomial comes from the greak word *nosos* meaning **disease** and *komeion* meaning **hospital.** Nintingale is the first person improves medical care in military and civilian hospital. Semmelweis and Holmes follow Nightingales medial care procedures to reduce the tramission of pathogenic microorganisms within hospital. Pasteur demonstrates that microorganism could be transmitted through air. In this time, Joseph Lister realizes the significance of Pasteur study and was convinced that microorganism in the air contaminates surgical wounds and he follows antiseptic surgery. Subsequently he also disinfects operating rooms greatly and reduces the mortality of surgical patients.

Rate of nosocomial infection

Nosocomial infections occur in approximately (one in 20 patients) 5 % of all patients admitted. Infection rate will vary depending on the type of hospital. Nosocomial infections in acute care institutions are follows. 40% of hospital infections are observed in urinary tract, 20% in surgical wound 10% in respiratory route. 5-10% in primary bacteria and others are due to opportunistic fungal and viral infections (20 – 250). Opportunistic infections are observed in immuno-compromised patients. Approximately 1% of all nosocomial infections directly cause death and 3% contribute to death.

Important pathogens of Nasocomial infections.

Staphylococcus aureus, Enterococcus, E. coli, Group B streptococci, Coagulase negative *Staphylococcus, Proteus, Pseudomonas aeruginosa,* Antibiotic resistant gram-negative rods, *Candida albicans, Torulopsis, Aspergillus, Anaerobic bacteria*

Sources of microbes that cause nosocomial infection:

There are two categories of infection.

Exogenous – caused by microbes from an external sources

Endogenous – caused by microbes that are part of a person's own normal flora.

Hospital personnel, Surgical procedures, Food, Air, Visitors, medications, Patients Normal Flora, Drains, implants, catherer, Other patients, Water, Fomites, Insects are the source of microbes in hospital.

Factors, which increase the risk of nosocomial infections

Invasive device, Tissue transplant , Extensive skin burn, Sickle cell anemia, Bone marrow failure, Malignant disorder, HIV infection, Malfunction of spleen, Implants.

The following precautions / isolation procedures should be taken to avoid hospital borne infection.

Table 3.1Methods to avoid Nosocomial infections

Strict isolation Diphtheria Smallpox Conjunctivitis Lassa fever Pneumonic plaque Chicken pox	**Contact isolation** Wound infections Gonococcus conjunctivitis Herpes simplex Influenza Pneumonia
Respiratory isolation Measles Meningitis Mumps Whooping cough pneumonia	**Enteric precautions** Amoebic dysentery Gastroenteritis Typhoid fever Cholera
Universal blood and body fluid precautions AIDS, HBV, Malaria , Syphilis Gonorrhoea	

Control of nosocomial infections

Because of the seriousness of nosocomial infections, the American Hospital Association (AHA) and the Centers for Disease Control (CDC) recommend that an each hospital develope an infection control programme. One of the most important activities of the control program is surveillance, the systematic observation and recording of cases of transmissible diseases. To accomplish this National Nosocomial Infection Surveillance system (NNIS) was established in 1970 by the CDC. In 1999 CDC update hospital control program for break cycle of infection. Methodologies are Hand washing, Aseptic technique, Isolation of an infected patient, Proper sanitation, Disinfection and Sterilization.

Components of control programme

1. Hospital policy-making committee - Develop policies for control of nosocomial infection
2. Infection control committee - Develop guidelines for patients care, monitor effectiveness of control program.
3. Microbiology laboratory - Isolate and identify organism, identify source of infection, monitor disinfection and sterilization process
4. Infection control officer -Implement infection control practices, surveillance and investigation of infection in patients and in personnel, maintain a continuing education program.

By combining importance of infection and control program Florence Nightingale said that ‘the very first requirement in a hospital is that it should do the sick no harm.

Check your progress

3.3. Is there any system is available in India to moniter nosocomial infection.

3.4. Who is called father of antiseptic surgery.

3.5. What is the role of CDC in the prevention of Nosocomial infection.

NOTES

3.10. LET US SUMUP

Indigenous microbes present in human body are called normal flora. Normal flora inhabits the skin and some of the inner parts of the body.

Skin is one of the most understandable regions in the body. It provides good barrier system and is considered as a first line defence system in the body. It has epidermis, dermis, subcutaneous connective tissue, sweat glands, sebaceous glands and hair follicles etc. Normal flora of the skin are *Coagulase negative – Staphylococcus, Staphylococcus aureus, Propionibacterium acnes, Bacillus sp., Streptococci, Malassezia furfur, Candida sp., Pityrosporum*

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Sources of microbes that cause nosocomial infection are person's own normal flora, Hospital personnel, Surgical procedures, Food, Air, Visitors, medications, Patients Normal Flora, Drains, implants, catherer, Other patients, Water, Fomites, Insects.

In 1999 CDC update hospital control program for break cycle of infection. Methodologies are Hand washing, Aseptic technique, Isolation of an infected patient, Proper sanitation, Disinfection and Sterilization.

3.11. UNIT END EXERCISES

Two Mark Questions

Define Normal flora
Resident flora
Transient flora
Give the general properties of normal flora
Listout the parts of human skin
Comedo
Mention the parts of respiratory track.
What are doderleins Bacilli
What is acne vulgaris
Define nosocomial infection

Five Mark Questions

Explain properties of human skin which discourage the growth of microorganisms.
Give the normal flora of skin.
What are the uses of Mucous?
Explain normal flora of teeth
Is normal flora is beneficial? Explain.
Why colon contain large number of normal flora
Colon is a large fermentation vessel? Why.
Explain risk factors and source of hospital borne infection
Write short note on nosocomial infection
List organisms responsible for hospital borne infection.
How do you control hospital borne infection.
Describe methods used to control hospital borne infection.

Ten Mark Question

Write an essay on normal flora of Healthy human
Describe hospital borne infection.

3.12. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

3.1. Colon is place for final food digestion and absorption; it is also accessible through anus. It also contains multiple quantities of waste nutrients.
3.2. It is because of mucous and alveolar macrophages.
3.3. Yes, National Nosocomial infection surveillance system, Ministry of health, government of India.
3.4. Joseph Lister
3.5. Setting up hospital infection control committee.

3.13. SUGGESTED READINGS

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BLOCK 2
BACTERIAL DISEASES

UNIT IV - GRAM POSITIVE & NEGATIVE COCCI DISEASE

Summary

4.1. INTRODUCTION

Bacteria are microscopic, unicellular, prokaryotic organism. Bacteria are the most abundant of all organisms. They are ubiquitous in nature. Bacteria present in this globe are morphologically distinct. Grams natures also differentiate bacteria on the basis of its cell wall. This unit illustrates diseases caused by gram positive and negative cocci.

NOTES

4.2. OBJECTIVES

Study of this unit will be able to

- Understand infections caused by *Streptococcus pyogenes, Streptococcus pneumonia* and *Neisseria gonorrhea*.
- Know the pathogenic mechanism of diseases like pharyngitis, pneumonia and gonorrhea.
- Helps in the process of laboratory diagnosis in pharyngitis, pneumonia and gonorrhea.
- Understand treatment of diseases caused by gram positive and negative bacteria.

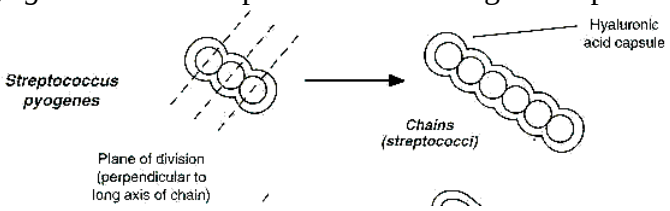
4.3. PHARYNGITIS

4.3.1. Introduction

Pharngitis is an inflammation of Pharynx. It is also called sore throat. Less severe form of sore throat is called strep throat. A painful condition of the throat is commonly called sore throat. It is due to inflammation of throat tissue. It is mediated by multiple factors. One among the reason is *Steptococcus pyogenes.*

4.3.2.Causative Agent

Steptococcus pyogenes is a Gram-positive cocci that grow in pairs and chains. Streptococci were first described by Billroth in 1874 in exudates from erysipelas and wound infections. Rosenbach (1884) isolated the cocci from human supportive lesions and gave the name *S. pyogenes*. It is also called Group A streptococci. It is a beta haemolytic Streptococci. *Streptococcus pyogenes* is a Gram positive, Spherical to ovoid in shape bacteria. It is a facultative anaerobic bacteria with fermentative metabolism. This organism is Catalase and oxidase negative.



4.3.3. Virulent factors of Steptococcus pyogenes

Hyaluranic acid (capsule) Prevent opsonization; Peptidoglycon (Induce fever, Cardiac necrosis); M Protein (Prevent phagocytosis); C_5a peptidase (Destroy chemotactic signals); Lipoteichoic acid (Improves bacterial colonization); Hemolytic hemolysin (Streptolysin O); Cytolytic hemolysin (Streptolysin S); Pyogenic exotoxin, Esterases, Streptokinase, Phosphatase, Proteinase, DNAase, ATPase, Nuclease

4.3.4. Pathogenesis

The pathogenicity of *Streptococcus pyogenes* (Group A) is multifactorial. A large number of virulent factors are produced by the pathogen during the infection. Organism enters into the human body by various direct and indirect mechanisms that are through droplets and

aerosols. From the portal of entry, organism adhere to epithelial cells via lipoteichoic acid and enhance the colonization. Once adherence has taken place, those strains that are able to resist phagocytosis and killing by leucocytes can proliferate and begin to invade local tissues.

Peptidoglycon also activate complement and induce inflammatory response. Pharyngeal infection may be symptomatic or may be associated with of Strep throat, Fever chills, Head ache, Malaise, Nausea and Vomiting. Pharynx may be mildly erythematous or beefly red with greyish yellow exudates and may be bleeding. From the throat, streptococci may spread to the surrounding tissues and to suppurative complications. It may also lead to meningitis.

The incubation period is typically two to five days. Symptoms that accompany pharyngitis are sore, dry, or scratchy throat, sneezing, runny nose, headache, cough, fatigue, body aches, chills, fever, Strep throat.

4.3.5. Lab Diagnosis
Sample - Throat swab, Nasopharyngeal aspiration, Blood.

Microscopy - A loop full of specimen is smeared on glass slide and fixed with methanol. Stain the smear with Gram staining technique. Smear is observed under high power magnification.

Culture - Most Streptococci grow aerobically and anaerobically. Temperature range for the growth is 22-42°C. Specimens are streaked on blood agar and incubated at 37°C. Colonies are less than 1mm, grey white or colourless, dry or shining and usually irregular. *Streptococcus pyogenes* produce β haemolytic colonies. Kanamycin blood agar is recommended for group B Streptococcus. When grown on serum starch agar *Streptococcus agalactiae* produces an orange pigment.

Serology
ASO Test - Anti Streptolysin O test, which is used to detect Streptolysin O in blood
Dick test - Erythrogenic toxin produce erythematous reaction in susceptible individual. For the detection of *S.pyogenes* 0.1ml of toxin is injected into human by intradermal injection and produce erythematosus reaction . This is called Dick test. Described by Dick in 1924.

Identification
Gram positive in chain, β haemolytic bacteria. Organism is sensitive toVancomycin, Bacitracin and resistant to SXT & Optochin. This organism reacted positively for PYR test and negatively for CAMP test, Hippurite hydrolysis, bile esculin hydrolysis and bile solubility test.

4.3.6.Treatment
Erythromycin, Clindamycin, Cephalexin, Penicillin, Vancomycin, Streptomycin .

4.3.7.Prevention

The best way to prevent sore throats is to avoid the germs that cause them and practice good hygiene.

- Wash hands thoroughly and frequently.
- Avoid sharing food, drinking glasses or utensils.
- Cough or sneeze into a tissue and throw it away.
- Use alcohol-based hand sanitizers as an alternative to washing hands.
- Avoid touching public phones or drinking fountains with your mouth.
- Regularly clean telephones, TV remotes and computer keyboards.
- Avoid close contact with people who are sick.

Check Your Progress

4.1. What are the predisposing factors of pharyngitis and Pnueumonia?

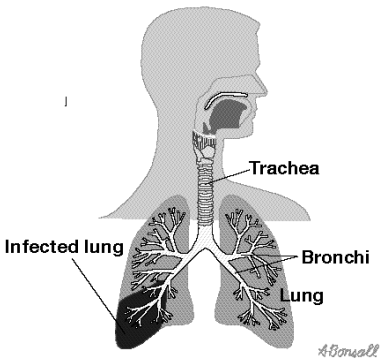
4.2. What is pharygitis

4.3. What is F-antigen or Forssman antigen

4.4. PNEUMONIA

4.4.1. Introduction

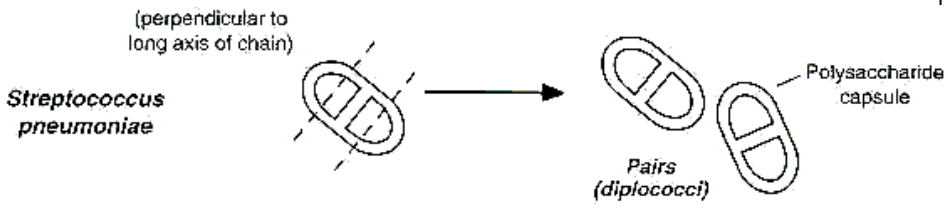
Pneumonia is an infection of one or both lungs which is usually caused by a bacteria, virus and fungus. It is an endogenous infection, because causative agents were contracted from normal flora of the respiratory tract. An infection of the lung that involves the small air sacs or alveoli and the tissue around them is called pneumonia.



PNEUMONIA

4.4.2. Causative Agents

Most common causative agent is *Streptococcus pneumoniae*. It causes epidemic or even pandemic form of pneumonia. It remains a leading cause of Morbidity and Mortality in humans of all ages. Currently, over 3 million people develop pneumonia each year in the United States.



*Streptococcus pne*umonia was first isolated by Pasteur and Sternbery independently during the year 1881 from human saliva.

It is a facultative anaerobic, Gram-positive coccus. It is also called Diplococcus, often called Pneumococcus. It is a Capsulated,

Nonmotile, Alpha hemolytic, Sometimes lancet shaped organism and able to ferment Inulin, solubilize bile.

4.4.3. Antigenic Structure of Causative Agent

Capsular antigen – it is made up of complex Polysaccharide that forms the hydrophobic gel on the surface of the organism. On the basis of capsular antigen 84 sero types were recognized.
Cellwall antigen – it is Species specific. Phosphocholine is a major antigenic determinant.

Galactosamine, glucose, phosphate, ribitol and trideoxydiamino hexoses are the other components.

F-antigen or Forssman antigen - This antigen cross-reacts with Forssman series of Mammalian cell surface antigen. It is lipoteichoic acid, consist of the C polysaccaride covalently linked to a lipid moiety. Distributed on the outer surface of the cellmembrane. Protein F is involved in attachment to fibronectin.

M-protein - It is type specific. It is like *S. pyogenes* M protein. M protein is a fibrillar surface protein. The M proteins are associated with both colonization and resistance to phagocytosis. M proteins have been identified on the basis of antigenic specificity.

4.4.4. Virulent Factors

The virulence is directly proportional to capsule antigen. Adhesions-Promote colonization. It binds specifically on N-acetyl hexosamine galactose polysaccaride component of cellwall. Capsule - It makes the pathogen resistant to phagocytosis and facilitate colonization. Neuraminidase - Attack glycoprotein and glycolipid component of cellmembrane. It cleaves the N-acetyl muramic acid residue of the cell wall
Protease - It specifically cleave the Immunoglobulin

4.4.5.Toxins

It is released during cell lysis. It is a 52.8 KDa thio activated cyto toxin. It binds with cholestrol component of cellmembrane and disturbs them by forming pores, thus kill the ciliated epithelial cells. It makes a contribution to the inflammatory response. It activates the complement pathway directly. It limits the phagocytic action of PMNs and Monocytes by decreasing oxidative burst. Hydrogen peroxide - It is produced during micro-aerophilic condition. It causes lung tissue damage. Lysozyme - It mediates autolysis of bacteria and release inflammatory cell materials.

4.4.6. Symptoms

Shortness of breath	Pain during inhalation
Fever	Pleural effusion
Chills	Abscess
Cough	Empyema
Chest pain	Rust coloured sputum

Complications

Local destruction of lung tissue	Ventilator dependence.
Frank cavitation	Pulmonary abscess.
Respiratory failure	Empyema
Respiratory distress syndrome.	Super infection
Bronchiectasis	Death

4.4.6. Pathogenesis

Everyone carries bacteria in their nose and throat without making them sick. In human beings about 80% of lobar pneumonia and 60% broncho pneumonia caused by *S. pneumoniae*. Death occurs about 1-3 days after onset of symptoms. Breathing of droplet nuclei allows the entry of bacteria to upper respiratory tract. It is inhibited by host defense mechanism. Pneumococci resist the defense mechanism and enters to the lungs, they usually settle in the air sac of the lung where they rapidly grow. During growth, capsule mediates colonization and also prevents phagocytosis

Peptidoglycon fragments and teichoic acid of cellwall activate the alternate pathway of complement and elicit the production of IL-1 and TNFα. As a result opsonin like C_3b and IgG, IgM antibodies are formed against cellwall antigens. These opsonins diffuse through the porous matrix of the capsule to the bacterial surface, where they bind and activate the classical complement pathway. This contributes the further production of C_5a. Complement activation attracts more PMNs but does not aid phagocytosis because C_3b and antibodies bound to cell wall. As a result of expanding inflammatory response tissue damage will occur, but it does not clear the bacteria.

Inflammatory response leads to blood vessel damage, this results in increase of vascular permeability, leakage of blood, vasodilation, extravagation, excess mucous secretion, accumulation of mucous, lung tissue damage, disturbance in gas exchange that leads to suffocation.
Damaging lung tissue not only provides the bacteria with a source of nutrients but also create a protected area to the bacterium. Then the bacteria spread throughout the body and create bacteremic condition and toxemic condition.

4.4.7. Laboratory Diagnosis

Specimen – Sputum, Throat swab

Processing of Specimen

Microscopy - Gram stain the specimen and observe under the high power objective lens. If diplococci with mirror image of the flame is observed. It indicates *Streptococcus pneumoniae* infection.

Macroscopy - Observe the colour and nature of the sputum. It is rust colour.

Culturing - Specimen is streaked on blood agar plate and incubate at 37°C for 24 hours under aeration. Alpha hemolytic colonies are observed. This organism solubilize bile.

4.4.8. Treatment

Cell wall inhibitors like Penicillin, Ampicillin are the drug of choice. Erythromycin, Chloramphenicol also used in some instances.

Check Your Progress

4.4. Why *Streptococcus pneumoniae* is called pnumococci.
4.5. Mention complications of Pneumonia
4.6. How capsule prevents host immune response
4.7. What is the nature of toxin produced by *S. pneumonia*

4.5. GONORRHEA

4.5.1. Introduction

Gonorrhea is a venereal disease. It has been known from ancient times. Galen first coined the name Gonorrhea in 130 A.D. The word Gonorrhea means *flow of seed.* The disease is acquired by sexual contact.

4.5.2. Causative Agent

Gonorrhea is caused by *Neisseria gonorrhoeae*. It is also called Gonococcus. Neisser first described it in gonorrheal pus in 1879. Bumm in 1885 cultured the coccus and proved its pathogenicity. It is a Gram negative coccus. It is a fastidious organism, 20% of strains require glutamine for primary isolation. It is an aerobic form and glucose is the principal carbon and energy source. Growth occurs best at pH 7.2-7.6 and at the temperature of 35 – 36°C. Some strains requires 5-10 % carbon-di-oxide. They grew well on chocolate agar and Mueller Hinton agar with 5% blood. A popular selective medium is the Thayer Martin Medium, the medium contains Vancomycin, Colistin, Nystatin and Trimethoprim lactate

Vancomycin inhibit Gram positive bacteria. Colistin inhibits Gram negative bacteria except *Proteus.* Nystatin inhibits yeast cells. Trimethoprime lactate inhibits *Proteus*. Colonies are small round, translucent, convex or slightly umbonate with finely granular surface and lobate margin. Four types of colonies have been recognized and named as T1, T2, T3 and T4. Gonococci only ferment glucose and not maltose. Gonococci are antigenically heterogeneous; they are capable of changing their surface structures. Gonococcus is a very delicate organism readily killed by heat, drying and antiseptics. It is highly susceptible to Sulfonamides, Penicillin etc.

4.5.3. Antigens

Pilin, Protein I, Protein II and Protein III, LOS, Tbp1, Tbp2, Lbp are the major antigens responsible for pathogenicity.

4.5.4. Pathogenesis

N. gonorrhoea is a fragile organism, which doesn't survive outside of a human host. It is only transmitted from person to person by

direct contact. It can colonize on mucosal surfaces and columnar epithelial cells of cervix and urethra. Within one hour after contact with the mucosal surface, the infection is established. During infection gonococci anchored with pili to surface of urethral cells. Lipopolysaccharide mediate colonization of bacteria, protein II intimate attachment, protein I prevent phagolysosome formation. At the same time LOS triggers inflammatory response. This is responsible for symptoms. Activities of inflammatory response lead to lysis of macrophages and form purulent discharge. Local production of TNFα elicited by LOS is through to be the main cause of tissue damage. Protein I make pore on the surface of macrophage and epithelial cells that also mediate tissue damage.

DISEASE IN MAN - An unprotected Male has an approximately 22% chance of acquiring Gonorrhea from intercourse with an infected women. Incubation period is about 2-8 days. The patient presents with burning on urination and yellow purulent discharge (urethral discharge) that indicates urethritis. This leads to urinary tract infection, sterilityand arthritis.

DISEASE IN WOMEN - About 20-80% of women with Gonorrhea is asymptomatic. Organism thrive in the cervices, fallopian tubes as well as in vaginal glands and other areas of female genital tract Symptomatic infections usually begins with painful urination and vaginal discharge. It leads to Pelvic Inflammatory Disease (PID). Scar tissue formed as a result of infection in fallopian tubes may block normal passage of ova through the tubes causing sterility. Scaring of fallopian tube can also lead to a dangerous complication - Ectopic pregnancy in which the ovum is fertilized and develops in the fallopian tube and leads to life threatening internal hemorrhaging. Sometimes Gonococcus cause Disseminated Gonococcal infection. The Gonococcus Arthritis- Dermatitis Syndrome is the most common manifestation. It is as a result of Gonococcal bacteremia. *Symptoms* are Fever, Chills, Malaiseand Arthritis

Opthalmia neonatorum is one of the eye infection occurs during childhood days. This disease is transmitted at birth from mother to infant during passage through a birth canal. Gonococcal vulvovaginitis occurs in girls at 2-8 years of age. The alkaline pH of prepubescent vagina is cited as one factor favoring the gonococcal disease in this age group.

45.5.Laboratory Diagnosis

Sample - Urethral discharge, Vaginal discharge and Blood

Gram staining - Stain smear with Gram staining technique. Observe bean shaped Gonococci inside of PMNs.

Culturing

Samples can be transported with the help of Stuart's medium and inoculated on Chocolate agar, Mueller Hinton agar, Thayer Martin agar and Newyork city agar medium. Incubate is the environment of 5-10% CO_2 at 35°C. Small grey, entire, sticky colonies formed on Chocolate agar. Serological tests are also available for the diagnosis of Gonorrhea.

4.5.6. Treatment

In 1989 CDC recommended a single dose of Ceftriaxone (250mg) intramuscularly plus Doxycycline (100mg orally for 7 days). Erythromycin (500mg) may be substituted for Doxycycline in pregnant womens.

Check Your Progress

4.8.What is gonococci

4.9. What is ectopic pregnancy

4.10. What is Opthalmia neonatorum

4.11. What is Gonococcal vulvovaginitis

4.12. Mention Complications Caused By Gonococci

4.6. LET US SUM UP

Pharyngitis

Pharngitis is an inflammation of Pharynx. It is also sore throat. It is due to inflammation of throat tissue. It is mediated by multiple factors. One among the reason is *Steptococcus pyogenes.Steptococcus pyogenes* is a Gram-positive cocci that grow in pairs and chains. Streptococci were first described by Billroth in 1874 in exudates from erysipelas and wound infections. *Streptococcus pyogenes* produce a large number of virulent factors. Organism enters into the human body, colonise and induce inflammatory response. Pharyngeal infections are associated with of Strep throat, Fever chills, Head ache, Malaise, Nausea and Vomiting. Pharynx may be mildly erythematous or beefly red with greyish yellow exudates and may be bleeding. The incubation period is typically two to five days. Most Streptococci grow aerobically and anaerobically. *Streptococcus pyogenes* produce Beta hemolytic colonies. Kanamycin blood agar is recommended for group B Streptococcus. Erythromycin, Clindamycin, Cephalexin, Penicillin, Vancomycin, Streptomycin are drug of choice. The best way to prevent sore throats is to avoid the germs that cause them and practice good hygiene.

Pneumonia

Pneumonia is an infection of one or both lungs which is usually caused by a bacteria, virus and fungus. Most common causative agent is *Streptococcus pneumoniae*. *Streptococcus pneumoniae* was first isolated by Pasteur and Sternbery. It is a facultative Gram-positive coccus. It is also called Diplococcus, often called Pneumococcus. It is a Capsulated, Nonmotile, Alpha hemolytic, Sometimes lancet shaped organism and able to ferment Inulin, solubilize bile. The virulence is directly proportional to capsule antigen. It binds specifically on N-acetyl hexosamine galactose polysaccaride component of cellwall. Toxins are released during cell lysis and kill the ciliated epithelial cells. It makes a contribution to the inflammatory response. Symptoms are Fever, chills, cough, chest pain, rust coloured sputum. Complication is local distruction of lung Tissue. Pneumococci resist the defense mechanism and enters to the lungs, they usually settle in the air sac of the lung where they rapidly grow. During growth, capsule mediate colonization and also

prevents phagocytosis. Inflammatory response leads to blood vessel damage, this results in increase of vascular permeability, leakage of blood, vasodilation, extravagation, excess mucous secretion, accumulation of mucous, lung tissue damage, disturbance in gas exchange that leads to suffocation. *Sputum and throat swab are used as specimen.*Gram stain the specimen and observe under the high power objective lens. If diplococci with mirror image of the flame is observed. It indicates *Streptococcus pneumoniae* infection. Specimen is streaked on blood agar plate and incubate at 37°C for 24 hours under aeration. Alpha hemolytic colonies are observed. This organism solubilize bile. Cell wall inhibitors like Penicillin, Ampicillin are the drug of choice. Erythromycin, Chloramphenicol also used in some instances

Gonorrhea

Gonorrhea is a venereal disease. Gonorrhea is caused by *Neisseria gonorrhoeae*. It is also called Gonococcus. Neisser first described it in gonorrheal pus in 1879. Bumm in 1885 cultured the coccus and proved its pathogenicity. It is a Gram negative coccus. It is a fastidious organism, 20% of strains require glutamine for primary isolation. It is an aerobic form and glucose is the principal carbon and energy source. It can colonize on mucosal surfaces and columnar epithelial cells of cervix and urethra. Within one hour after contact with the mucosal surface, the infection is established. During infection gonococci anchored with pili to surface of urethral cells. Lipopolysaccharide mediate colonization of bacteria, protein II intimate attachment, protein I prevent phagolysosome formation. At the same time LOS triggers inflammatory response. This is responsible for symptoms. Activities of inflammatory response lead to lysis of macrophages and form purulent discharge. Local production of TNFα elicited by LOS is through to be the main cause of tissue damage. Protein I make pore on the surface of macrophage and epithelial cells that also mediate tissue damage. Incubation period is about 2-8 days. The patient presents with burning on urination and yellow purulent discharge (urethral discharge) that indicates urethritis. This leads to urinary tract infection, sterilityand arthritis. About 20-80% of women with Gonorrhea is asymptomatic. Organism thrive in the cervices, fallopian tubes as well as in vaginal glands and other areas of female genital tract Symptomatic infections usually begins with painful urination and vaginal discharge. It leads to Pelvic Inflammatory Disease (PID). Opthalmia neonatorum is one of the eye infection occurs during childhood days. Gonococcal vulvovaginitis occurs in girls at 2-8 years of age. Samples can be transported with the help of Stuart's medium and inoculated on Chocolate agar, Mueller Hinton agar, Thayer Martin agar and Newyork city agar medium. In 1989 CDC recommended a single dose of Ceftriaxone (250mg) intramuscularly plus Doxycycline (100mg orally for 7 days).

4.7. UNIT END EXERCISES

Two Mark Questions

Pharyngitis
Sorethroat
Strepthroat

ASO test
Dike test
Pneumococci
Diplococcus
Gonococci
Define PID
Ectopic pregnancy

Five Mark Questions

Give the characters of *Streptococcus pyogenes*
Listout virulent factors of *Streptococcus pyogenes*
Give the symptoms associated with Pharyngitis
How do you prevent Pharyngitis
Explain pathogenesis of pneumonia caused by *S. pneumonia.*
Give the virulent properties of *S. pneumonia.*
Describe lab diagnosis of Pneumonia caused by *S. pneumonia.*
What are the complications caused by pneumonia caused by *S. pneumonia.*
Describe pathogenesis caused by Gonococci.
Explain Lab diagnosis and treatment of Gonorrhoea.

Ten Mark Questions

Write an essay on pharyngitis.
Give a detailed note on Pnumonia of *S. pneumoniae.*
Describe salient features of Gonorrhoea.

4.8. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

4.1. Predisposing factors of respiratory infections like pharyngitis and pneumonia are air pollution, smoking, use of alcohol, source of pathogenic microorganisms.

4.2. Pharyngitis is a inflammatory condition caused by various microorganisms like Streptococcus, Staphylococcus.

4.3. This antigen cross-reacts with Forssman series of Mammalian cell surface antigen. It is lipoteichoic acid, consist of the C polysaccaride covalently linked to a lipid moiety. Distributed on the outer surface of the cellmembrane. Protein F is involved in attachment to fibronectin.

4.4. It is because this diplococcus causes pneumonia and called pneumococci.

4.5. Mention complications of Pneumonia

Respiratory distress, Ventilator dependence, Pulmonary abscess, Empyema, Super infection

4.6. It prevent phagolysosome formation

4.7. Toxin of *S. pneumonia is* cytotoxic in nature

4.8. Gonococci is a gram negative organism capable of causing Gonorrhoea.

4.9. Ectopic pregnancy is a condition in which the ovum is fertilized and develops in the fallopian tube instead of uterus and leads to life threatening internal hemorrhaging.

4.10. It is one of the eye infection caused by gonococci

4.11. It is one of the inflammation of genetal track caused by gonococci. It occurs in girls at 2-8 years of age.

4.12. Disseminated Gonococcal infection, Sterility and The Gonococcus Arthritis- Dermatitis Syndrome.

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NOTES

NOTES

UNIT-V GRAMPOSITIVE NON SPORE& SPOREFORMING BACILLI

Summary

5.1 INTRODUCTION

Bacteria are unique group of microorganisms. Gram positive microorganisms are more resistant than gram negative bacteria. These

organisms showed higher G+C Content. Spore forming microorganisms are found in gram positive microorganisms. This unit describes diseases of gram positive sporeforming and non sporeforming gram positive bacteria.

5.2 OBJECTIVES

NOTES

Studying this unit will be able to

Differentiate spore forming non spore forming gram positive bacteria.

Understand major diseases caused by Gram positive bacteria.

Understand importance of lab diagnosis procedure.

Describe aetiology, symptoms, pathogenesis, treatment and prevention of diphtheria, tetanus, anthrax.

5.3. NOCARDIOSIS

5.3.1. Introduction

Nocardiosis is an acute, subacute or chronic infectious disease. It occurs in cutaneous, pulmonary and disseminated forms. Primary cutaneous nocardiosis manifests as cutaneous infection (cellulitis or abscess), lymphocutaneous infection (sporotrichoid nocardiosis), or subcutaneous infection (actinomycetoma). Pleuropulmonary nocardiosis manifests as an acute, subacute, or chronic pneumonitis, usually in immunocompromised hosts, although isolated cases have been reported in immunocompetent hosts. Disseminated nocardiosis may involve any organ; lesions in the brain or meninges are most common.

5.3.2. Causative agent

Nocardia is a genus of weakly staining Gram-positive, catalase-positive, rod-shaped bacteria. It is a obligate aerobic, partially acid-fast, beaded, branching bacilli. Several *Nocardia* sp, in the family Actinomycetaceae, cause human disease. Some species are nonpathogenic, while others are responsible for nocardiosis.

Nocardia species are found worldwide in soil rich in organic matter. In addition, they are oral microflora found in healthy gingiva, as well as periodontal pockets. Most Nocardia infections are acquired by inhalation of the bacteria or through traumatic introduction. *N. asteroides* is the most common human pathogen; it usually causes pulmonary and disseminated infection. *N. brasiliensis* most commonly causes skin infection, particularly in tropical climates. Infection is via inhalation or by direct inoculation of the skin. Other *Nocardia* sp sometimes cause localized or, occasionally, systemic infections. Nocardiosis occurs worldwide in all age groups, but incidence is higher in older adults, especially menand immunocompromised patients. Person-to-person spread is rare.

Nocardia colonies have a variable appearance. Nocardia grow slowly on nonselective culture media and are strict aerobes with the ability to grow in a wide temperature range. Some species are partially acid - Fast due to the presence of intermediate-length Mycolic acids in their cell wall. Majority of strains possess the cord

factor (trehalose 6-6' dimycolate), an important virulence factor. They are catalase positive and can grow easily on the most commonly used media with colonies becoming evident in 3–5 days.

5.3.3. Virulence

It contains catalase, superoxide dismutase and "cord factor" (which interferes with phagocytosis).

5.3.4. Signs and Symptoms

The symptoms of nocardiosis vary depending on which part of your body is affected. Nocardiosis most commonly occurs in the lungs. Fever, Weight loss, Night sweats, Cough, Chest pain, Pneumonia. When lung infections occur, the infection can spread to the brain.

5.3.5. Pathogenesis

Skin infections can occur when soil containing Nocardia species gets into open wounds or cuts. Farming or gardening without gloves and protective clothing increases the risk of cuts, thorn pricks, or other minor injuries. Skin ulcers, Nodules, sometimes draining, with the infection spreading along lymph nodes

5.3.6. *Lab Diagnosis*

Diagnosis of nocardiosis can be made by a chest x-rays of the lung, a bronchoscopy, a brain/lung/skin biopsy, or a sputum culture. Nocardiae are gram positive, weakly acid-fast, branching rod-shaped bacteria and can be visualized by a modified Ziehl–Neelsen stain such as the Fite-Faraco method. In the clinical laboratory, routine cultures may be held for insufficient time to grow nocardiaeand referral to a reference laboratory may be needed for species identification. Pulmonary infiltration and pleural effusion are usually detected via x-ray.

5.3.7. *Treatment*

Nocardiosis requires at least 6 months of treatment, preferably with trimethoprim/sulfamethoxazole or high doses of sulfonamides. In patients who do not respond to sulfonamide treatment, other drugs, such as ampicillin, erythromycin, or minocycline, may be added. A new combination drug therapy (sulfonamide, ceftriaxoneand amikacin) has also shown promise.

Check Your Progress

5.1. What is Nocardia cord factor
5.2. Name the virulent factors of Nocardia
5.3. Is Nocardia is a true bacteria
5.4. Name the staining used to stain nocardia
5.5. Mention name of skin infection caused by Noccardia

5.4. DIPHTHERIA

5.4.1. Introduction

CDC describes diphtheria as "an upper respiratory tract illness characterized by sore throat, low-grade feverand an adherent membrane of the tonsil(s), pharynxand/or nose". Diphtheria is a rapidly developing,

acute, febrile infection, which involves both local and systemic pathology. A local lesion develops in the upper respiratory tract and involves necrotic injury to epithelial cells. As a result of this injury, blood plasma leaks into the area and fibrin network forms, which is, interlaced with rapidly growing *C. diphtheriae* cells. This membranous network covers over the site of the local lesion and is referred to as the **pseudomembrane**.

5.4.2. Causative agent

The bacterium that cause Diphtheria was first described by Klebs in 1883and was cultivated by Loeffler in 1884, who applied Koch's postulates and properly identified *Corynebacterium diphtheriae* as the agent of the disease. In 1884, Loeffler concluded that *C. diphtheriae* produced a soluble toxinand thereby provided the first description of a bacterial exotoxin. In 1888, Roux and Yersin demonstrated the presence of the toxin in the cell-free culture fluid of *C. diphtheriae* which, when injected into suitable lab animals, caused the systemic manifestation of diphtheria. In 1913, Schick designed a skin test called **The Schick Test.** It involves injecting a very small dose of the toxin under the skin of the forearm and evaluating the injection site after 48 hours. A positive test (inflammatory reaction) indicates susceptibility (nonimmunity). A negative test (no reaction) indicates immunity (antibody neutralizes toxin).

Corynebacteria are Gram-positive, aerobic, nonmotile, rod-shaped bacteria related to the Actinomycetes. They do not form spores or branch as do the Actinomycetes, but they have the characteristic of forming irregular shaped, club-shaped or V-shaped arrangements in normal growth. They undergo snapping movements just after cell division, which brings them into characteristic arrangements resembling Chinese letters.

Three strains of *Corynebacterium diphtheriae* are recognized by Mc.Leod on the basis of growth and other characters , gravis, intermedius and mitis. The gravis strain has a generation time (in vitro) of 60 minutes; the intermedius strain has a generation time of about 100 minutes; and the mitis strain has a generation time of about 180 minutes. The faster growing strains typically produce a larger colony on most growth media.

5.4.3. Mode of Transmission

Transmission occurs by droplet spread through contact with a patient or carrier, or articles soiled with discharges from infected lesions.

Incubation Period - Usually two to five days.

5.4.4. Pathogenesis

Humans are the primary reservoir for *C. diphtheriae*. Sources of infection include carriers who have recovered from infection. The organisms are inhaled and establish infection in the upper respiratory tract usually in the nose or throat. Diphtheria bacilli do not tend to invade

tissues below or away from the surface of the epithelial cells at the site of the local lesion. At this site they produce the toxin that is absorbed and disseminated through lymph channels and blood to the susceptible tissues of the body. Degenerative changes in these tissues, which include heart, muscle, peripheral nerves, adrenals, kidneys, liver and spleen, result in the systemic pathology of the disease.

The pathogenicity of *Corynebacterium diphtheriae* includes **two distinct phenomena**: **Invasion** of the local tissues of the throat, which requires colonization and subsequent bacterial proliferation. **Toxigenesis**: The diphtheria toxin causes the death of eukaryotic cells and tissues by inhibition of protein synthesis. Although the toxin is responsible for the lethal symptoms of the disease, the virulence of *C. diphtheriae* cannot be attributed to toxigenicity alone, since a distinct invasive phase apparently precedes toxigenesis.

Toxigenicity

Two factors have great influence on the ability of *Corynebacterium diphtheriae* to produce the diphtheria toxin: 1. Low extracellular concentrations of iron (2) The presence of a lysogenic prophage in the bacterial chromosome.

The role of iron. In artificial culture the most important factor controlling yield of the toxin is the concentration of inorganic iron (Fe++ or Fe+++) present in the culture medium.

The role of β-phage. Only those strains of *Corynebacterium diphtheriae* that are lysogenized by a specific Beta-phage produce diphtheria toxin.

Mode of Action Of The Diphtheria Toxin

The diphtheria toxin is a two component bacterial exotoxin synthesized as a single polypeptide chain containing an A (active) domain and a B (binding) domain. Proteolytic nicking of the secreted form of the toxin separates the A chain from the B chain

The toxin binds to a specific receptor (now known as the HB-EGF receptor) on susceptible cells and enters by Receptor-Mediated Endocytosis. Acidification of the endosome vesicle results in unfolding of the protein and insertion of a segment into the endosomal membrane. Apparently as a result of activity on the endosome membrane, the A subunit is cleaved and released from the B subunit as it inserts and passes through the membrane. Once in the cytoplasm, the A fragment regains its conformation and its enzymatic activity. Fragment A catalyzes the transfer of ADP-ribose from NAD to the eukaryotic Elongation Factor 2 which inhibits the function of the latter in protein synthesis. Ultimately, inactivation of all of the host cell EF-2 molecules causes death of the cell.

5.4.5. Symptoms

Acute bacterial disease of tonsils, pharynx, larynx, nose and occasionally other mucous membranes, skin, conjunctiva and genitalia.

The throat is sore in faucial or pharyngotonsillar diphtheria with cervical lymph nodes enlarged and tender. In severe cases, there is marked swelling and oedema of the neck. Cutaneous diphtheria also occurs, generally without systemic symptoms. Laryngeal disease is serious in infants and young children. Case fatality rate is 5-10 per cent for non-cutaneous diphtheria.

5.4.6. Lab diagnosis

Sample: Nasopharyngeal swab should be obtained with a flexible alginate swab that reaches deep into the posterior nares.

Whitish membrane on tonsils is the major clinical diagnosis

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Loefler serum medium, Dextrose Proteose peptone agar, Hoyle medium, Tinsdale agar, potassium tellurite agar are used for cultivation. Sample is inoculated on loeflers serum slope and incubated for 48hours and subcultured on potassium tellurite agar. *C. diptheriae* grown as black coloured colonies.

5.4.7. Treatment

Administration of antitoxin is the most important fact of treatment and must not be delayed. Antibiotics are commonly used are Oral erythromycin, IM penicillin and Benzathine penicillin (single dose).

5.4.8. Prevention

Diphtheria toxoid is recommended for all persons at two, four, six, 18 months and five years (given as DPT (Triple Antigen)) and at 15 years and every 10 years thereafter (given as ADT). Health workers and those at high risk of patient exposure should be fully immunised and receive 10-year boosters. Currenty pentavalent vaccine is given in the name of Indirathanush in India.

Check Your Progress

5.6. Name the bacteria responsible for Psudomembrane formation.

5.8. Why Pseudomembrate is formed in Diphtheria infection.

5.9. Name important media used for *C. dipththeriae* cultivation.

5.10. Mention strains of *C. dipththeriae.*

5.5. ANTHRAX

5.5.1. Introduction

Anthrax is a rare and serious infectious disease. It mainly affects livestock and wild animals. Humans can become infected through direct or indirect contact with sick animals. It is a zoonotic disease. It is not a contagious disease.

5.5.2.Causative agent

It is caused by gram-positive, rod-shaped bacteria known as *Bacillus anthracis*. This bacterium is found naturally in soil. Cells of *Bacillus anthracis* are large square ended spore forming bacillus. It is also a capsulated organism. Capsule is made up of D-Glutamic acid.

Spores are oval and central or subterminal in position. Spores are formed only in soil. The capsules are formed in the tissue. It is a aerobic bacterium grows in ordinary medium at 37°C. Grows well in 5% sheep blood agar, chocolate agar, Phenylethylalcohol agar. Presence of bicarbonate in medium induce capsule formation. PLET medium (Polymyxin, Lysozyme, EDTA, Thallous acetate in heart infusion agar) is used to isolate *Bacillus anthracis* from mixed spore forming bacteria. *Bacillus anthracis* ferment glucose, sucrose and maltose with acid only. It is a catalase positive organism and reduce nitrate to nitrite.

On sheep blood agar medium colonies are round non haemolytic in nature. Edge of the colonies showed wavy margin and radiating projections. This is because edge of the colony is composed of long interlacing chain of bacilli. These types of colony are described as medusa head colonies.

5.5.3. Virulence factors

Virulence of *Bacillus anthracis* is due to two different factors. They are exotoxin and capsular polypeptide. These two virulence are coded by plasmids pXO1 and pXO2. Exotoxin consists of 3 components, they are the oedema factor, the protective antigen and the lethal toxin. Mixture of these three factors able to cause toxic effect (Anthrax toxic complex). The capsule inhibits opsonization and phagocytosis.

5.5.4. Pathogenesis

Anthrax is a primary disease to sheap, goat and cattles. Human infects accidentally. After entry, the spores germinate in to vegetative form and produce toxins. Receptors of the protective antigen is available in more tissues, hence a large range of tissues can be damaged by anthrax toxin. There are three types of anthrax based on its entry. They are cutaneuous anthrax, pulmonary anthrax and intestinal anthrax. Cutaneous anthrax is most common in human. Spores enter through skin abrasions or hair follicle of wound. Common sites of infections are face, neck, arms and back. Incupation period is 2-5 days. The lesions are surrounded by an area of edema and erythema due to multiplication of organisms. The lesion is converted to vesicles of 1-3 cm diameter. The fluid in the vesicle are turns black due to hemorrhage. The lesion finally ulcerates and develops a black buttoned eschar with surrounding oedema. This is called malignant pustule.

Pulmonary anthrax is due to inhalation of dust, wool from animal contains spores causes this type of anthrax. It is also called woolsorters disease. Incubation period is 1-3 days. It is responsible for death in bioterrorism attack. Spores are phagocytosed by macrophages and carried to mediastinal lymphnodes & germinates, cause hemorrhagic lymphadenitis. Toxins of organism causes inflammatory response in trachea, bronchioles leads to hemorrhagic bronchopneumonia and septicaemia. Fatality rate is 80-90%.

Intestinal anthrax is rare in human. It is due to ingestion of spore contaminated meat. Incubation period is 2-7 days. Severe enteritis with

NOTES

bloody diarrhoea is a condition of this disease with 98% fatality. Death occure within 2-8 days.

5.5.5. Symptoms

The symptoms of anthrax depend on the type of infection and can take anywhere from 1 day to more than 2 months to appear. All types of anthrax have the potential, if untreated, to spread throughout the body and cause severe illness and even death.

Cutaneous anthrax symptoms are a group of small blisters or bumps that may itch, Swelling can occur around the sore, A painless skin sore (ulcer) with a black center that appears after the small blisters or bumps.

Inhalation anthrax symptoms are fever and chills, chest Discomfort, Shortness of breath, Confusion or dizziness, Cough, Nausea, vomiting, or stomach pains, Headache, Sweats, Extreme tiredness and Body aches.

Gastrointestinal anthrax symptoms are Fever and chills, Swelling of neck or neck glands, Sore throat, Painful swallowing, Hoarseness, Nausea and vomiting, especially bloody vomiting, Diarrhea or bloody diarrhea, Headache, Flushing (red face) and red eyes, Stomach pain, Fainting, Swelling of abdomen (stomach)

5.5.6. Lab Diagnosis

Pulmonary anthrax is diagnosed by chest X-rays or CT scans. The only ways to confirm an Anthrax diagnosis are to measure antibodies or toxin in blood and to isolate *Bacillus anthracis* directly from the sample.

Sample – blood, skin lesion swab, spinal fluid, throat swab, nasopharyngeal aspirations, sputum. Samples must be taken before the patient begins taking antibiotics for treatment.

Organisms grow on sheep blood agar with medusa head appearance. Organism is gram positive, motile, blunt end bacilli in nature. It is spore positive, capsule positive. It also showed positive to MFadyeans reaction. It is performed by staining blood film with polychrome methylene blue for 10-20 seconds. *Bacillus anthracis* appear as blue bacilli surrounded by a red granular stained capsule.

Bacillus anthracis does not produce turbidity in broth. Gelation is liquefied slowly and appear as inverted fir tree appearance. Charcoal hydrate inhibits growth of *Bacillus anthracis. Bacillus anthracis* ferments salicin and is susceptible to gamma phage.

5.5.7. Treatment

Antibiotics are recommended to prevent infection in anyone exposed to the spores. Ciprofloxacin (Cipro), doxycycline (Monodox, Vibramycin, others) and levofloxacin (Levaquin) are approved by the

Food and Drug Administration for post-exposure prevention of anthrax in adults and children.

5.5.8. Prevention

An anthrax vaccine for humans is available. The vaccine doesn't contain live bacteria and can't lead to infection. Safe handling of infected animal. Proper hygienic measures.

Check Your Progress

5.11. What is wool sorters disease.

5.12. Name the spore produced by Bacillus anthrasis.

5.13. What is Medusa head.

5.14. What is MFadyeans reaction.

5.6. TETANUS (LOCK JAW).

5.6.1. Introduction

The word Tetanus comes from the Greek word tetanos, which means “to stretch”. This disease originates from wound and selectively impairs the activity of inhibitory chemicals that control the nerve impulse conduction. This disease is world wide in distribution. Tetanus has been known from very ancient times, having been described by Hippocratus. Arthur Nicholaire discovered the Tetanus bacillus in 1884. The organism was isolated in pure form by Kitasato in 1889.Tetanus results from the infection of a wound or raw surface with Tetanus bacilli. Most frequently this disease follows injury. Puncture wounds are particularly vulnerable as they favor the growth. Rarely it may follow surgical operation, usually due to lapses in asepsis. Sometimes the disease may be due to suppurations, such as otitis media. It is one of the complications in septic abortion. Tetanus may also be caused by unsterile injection.

It was a serious disease with a high rate of mortality, 80- 90% death observed before the start of treatment. Even with proper treatment the case fatality rate varies from 15-50%.

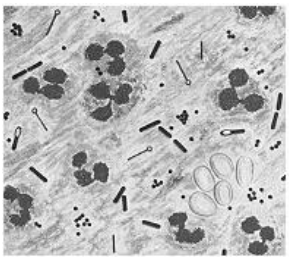
Tetanus is more common in the developing countries with warm climate and rural areas where the soil is fertile and highly cultivated. In India tetanus is estimated to be 4^{th} commonest cause of death. It is world wide in distribution. Unimmunized rural population develops Tetanus at higher rates than urban population. The disease is very low in well-developed countries.

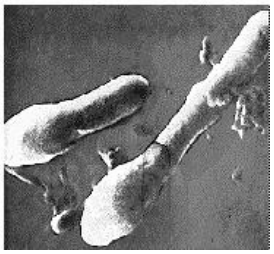
5.6.2. Causative Agent

Tetanus is caused by *Clostridium tetani*. It is quite long and thin organism. It is usually Gram positive and produce terminal spore , so it appears like drumstick. Motility by Peritrichous flagella. Obligate anaerobe

Optimum temperature 37°C and pH 7.4. organism is moderately fastidious requires vitamins and amino acids for growth. Noncapsulated. It grows in Robertson Cooked Meat Medium with turbidity and some gas formation within 48 hours, there is no digestion of meatand black colour

is formed during prolonged incubation. It doesn't ferment any carbohydrate. In horse blood agar alpha hemolysis is observed first, then beta hemolysis is observed due to prolonged incubation and production of hemolysins. Organism is Indole positive, Methyl red and Voges Proskauer negative, Gelatin liquefaction occurs very slowly. It is resistant to disinfections and withstand heat. Flagella, somatic antigens have been demonstrated. Ten types of organisms were identified on the basis of flagellar antigens and have a single type of somatic antigen. Type IV is non-flagellated organism. Organism produce 2 distinct toxins. They are Tetanolysin and Tetanospasmin





Tetanolysin - It disturb the activity of RBC and WBC, It is oxygen labile and heat labile.

Tetanospasmin - It is responsible for the symptoms of tetanus. Toxins are released during autolysis of cell. Heat labile protein inactivated by heating for 20 minutes at 60°C. Its molecular weight is about 150,000 daltons. It has two non identical polypeptide chain having molecular weight of 93,000 and 52,000, both held together by disulphide bridges. It selectively binds ganglioside. Gangliocide binding region found on heavy chain region whereas light chain is responsible for toxic activity.

5.6.3. Pathogenesis

The spores of *Clostridium tetani* are ubiquitous in nature. They have been found in 20-64% of soil samples. Spores are generally implanted at the part of contamination. Under any favorable conditions bacilli germinates. Organism has little invasive power. Organism alone does not cause disease. It requires the help of aerobic bacterium, which is responsible for removal of oxygen and creation of anaerobic environment. After implantation of spores in to an appropriate environment it cause localized infection and later it cause generalized infection. After specific incubation period (4-10 days) toxins are released from the cell by autolysis mechanism. Toxin is observed and spread throughout the body and cause various complications. Toxins take up by internalization and transport intra axanally against flow. When it reaches the region of nucleus it is transported to the interneurons where it inhibits the inhibitory transmitters.

5.6.4. Symptoms

The earliest manifestation is muscle stiffness followed by the spasm of the master muscles, lockjaw. As the disease progress, it causes, Clenching of the jaw, Arching of the back, Flexion of the arms, Hypertension, Hypotension, Cardiac disturbance, Respiratory problems and Bone fractures.

5.6.5. Epidemiology

C. tetani can be isolated from the soil in almost every environment throughout the world. The organism can be found in the gastrointestinal flora of humans, horsesand other animals. Isolation of *C. tetani* from the intestinal flora of horses, coupled with the high frequency of equine tetanus, led to the erroneous assumption that the horse was the animal reservoir of *C. tetani*.

5.6.6. Lab Diagnosis

Specimen : Wound exudate and pus

Microscopic examination is useful to demonstrate the presence of bacilli Gram staining is performed. Cultivation is with the help of Robertson Cooked Meat Medium. Based on the Saccharolytic and Proteolytic reactions all Clostridias are differentiated.

Saccharolytic	-	Red colour formation
Proteolytic	-	Black colour formation

5.6.7. Control

The nature of prophylaxis depends largely on the type of wound and the immune status of the patient. The available methods are Surgical attention, Antibiotics and Immunization

Surgical prophylaxis aims at removal of foreign bodies, necrotic tissue and blood clots to avoid providing an anaerobic environment favourable for Tetanus bacillus.

Injections of Tetanus toxoid are prophylactic. Currently, booster doses are recommended only every 10 years by the CDC. An antibody titer above 0.01 international units (IU) per ml is usually considered protective. Human Tetanus Immunoglobulin (HTIG) in a dose of 250 IU intramuscularly.

Routine immunization with Tetanus toxoid should begin at 1-3 months of age by DPT vaccine. Three doses of DPT given at intervals of 3-4 weeks, with booster doses 1 and 4 years later. Immunity to Tetanus can be maintained by a single booster dose of toxoid at every 10 years.

Check Your Progress

5.15. Who is Arthur Nicolaire

5.16. Who isolated *Clostridium tetani* as pure culture.

5.17. What is Peritrichous flagella

5.18. Which medium is used for the cultivation of *Clostridium tetani*

5.19. What is the mode of action of tetanospasmin

5.7. LET US SUM UP

Nocardiosis is an acute, subacute, or chronic infectious disease. It occurs in cutaneous, pulmonaryand disseminated forms. Most Nocardia infections are acquired by inhalation of the bacteria or through traumatic introduction. N. asteroides is the most common human pathogen; it usually causes pulmonary and disseminated

infection. N. brasiliensis most commonly causes skin infection, particularly in tropical climates. Infection is via inhalation or by direct inoculation of the skin. Nocardia grow slowly on nonselective culture mediaand are strict aerobes with the ability to grow in a wide temperature range. Some species are partially acid-fast due to the presence of intermediate-length mycolic acids in their cell wall. Majority of strains possess the cord factor (trehalose 6-6' dimycolate), an important virulence factor. Fever, Weight loss, Night sweats, Cough, Chest pain, Pneumonia. When lung infections occur, the infection can spread to the brain. Diagnosis of nocardiosis can be made by a chest x-rays of the lung, a bronchoscopy, a brain/lung/skin biopsy, or a sputum culture. Nocardiosis requires at least 6 months of treatment, preferably with trimethoprim/sulfamethoxazole or high doses of sulfonamides.

Diphtheria is an upper respiratory tract illness characterized by sore throat, low-grade feverand an adherent membrane of the tonsil(s), pharynxand/or nose. It causes localized blood vessel injury, blood plasma leaks into the area and a fibrin network forms and is referred to as the pseudomembrane. Three strains of Corynebacterium diphtheriae are gravis, intermedius and mitis. The pathogenicity of *Corynebacterium diphtheriae* includes two distinct phenomena: Invasion of the local tissues of the throat, which requires colonization and subsequent bacterial proliferation. Toxigenesis: The diphtheria toxin causes the death of eukaryotic cells and tissues by inhibition of protein synthesis. Loefler serum medium, Dextrose Proteose peptone agar, Hoyle medium, Tinsdale agar, potassium tellurite agar are used for cultivation. Administration of antitoxin is the most important fact of treatment. Antibiotics are commonly used are Oral erythromycin, IM penicillin and Benzathine penicillin (single dose). Diphtheria toxoid is recommended for all persons at two, four, six, 18 months and five years (given as DTP (Triple Antigen)).

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Tetanospasmin. Spores are generally implanted at the part of contamination. The earliest manifestation is muscle stiffness followed by the spasm of the master muscles, lockjaw. As the disease progress, it causes, Clenching of the jaw, Arching of the back, Flexion of the arms, Hypertension, Hypotension, Cardiac disturbance, Respiratory problems and Bone fractures. Microscopic examination is useful to demonstrate the presence of bacilli Gram staining is performed. Cultivation is with the help of Robertson Cooked Meat Medium. The available methods are Surgical attention, Antibiotics and Immunization. Surgical prophylaxis aims at removal of foreign bodies, necrotic tissue and blood clots to avoid providing an anaerobic environment favourable for Tetanus bacillus. Injections of Tetanus toxoid are prophylactic.

5.8. UNIT END EXERCISES

Two Mark Questions

- Nocardiasis
- Listout virulent proprtties of Nocardia.
- Schick Test
- Tetanospasmin
- Tetanolysin
- PLET Medium
- Medusa head

Five Mark Questions

- What is the mode of action of tetanus toxin
- Robertson Cooked meat medium
- Give the prophylaxis of Tetanus infection
- Describe General characters of Clostridium tetani
- Explain laboratory diagnosis of tetanus
- Explain mode of action of diphtheria toxin.
- What is Woolsorters disease.
- Explain the features of anthrax toxin

Ten Mark Question

- Write a detailed note on Tetanus
- Write an essay on Diphtheria.
- Give a brief note on Nocardiasis
- Explain seriousness of Anthrax

5.9. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

5.1. Trehalose 6, 6 Dimycolate is calle cord factor. It is responsible for virulence.
5.2. Catalase, superoxide dismutase and cord factor.
5.3. No, It is a Actinobacteria.
5.4. Acid Fast staining
5.5. Actinomycetoma
5.6. Corynebacteriul diphtheriae
5.8. Diphtheria Toxins causes tissue and capillary damagein respiratory track, which leads of blood leakage. This blood clotted in that area which is noted as membrane.
5.9. Loefller serum slope, Hoyle medium.
5.10. Gravis, intermedius and Mitis

5.11. Anthrax is also called wool sorters disease.
5.12. Cebtral spore.
5.13. On sheep blood agar medium colonies are round non haemolytic in nature. Edge of the colonies showed wavy margin and radiating projections. This is because edge of the colony is composed of long interlacing chain of bacilli. These types of colony are described as medusa head colonies
5.14. It is performed by staining blood film with polychrome methylene blue for 10-20 seconds. *Bacillus anthracis* appear as blue bacilli surrounded by a red granular stained capsule.
5.15. He discovered *Clostridium tetani*.
5.16. Kitasatto
5.17. Presence of flagella throuout the cell.
5.18. Robertson cooked meat medium
5.19. Tetanospasmin is responsible for the symptoms of tetanus. Toxins are released during autolysis of cell

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UNIT-VI GRAM NEGATIVE NONSPORE FORMING BACILLI

Summary

6.1 INTRODUCTION

Bacilli present in this group are responsible for numerous diseases. Some are commensal organisms present among normal intestinal flora. These commensal organisms plus others from animal or environmental reservoirs may cause disease.

6.2 OBJECTIVES

After reading this learners will be able to

Understand the concept pertusis

Express disease causing mechanism of gram negative bacilli

Understans causative nature, lab diagnosis of pertusis and Yersinia infection.

6.3. PERTUSSIS

6.3.1. Introduction

Pertussis, or Whooping cough, is a highly contagious disease involving the respiratory tract. It is caused by a bacterium that is found in the mouth, nose and throat of an infected individual. Pertussis means

"Intensive Cough". Pertussis is primarily spread by direct contact with discharges from the nose and throat of an infected individual.

6.3.2. Causative Agent

This disease is caused by *Bordetella pertussis. Bordetella pertussis* was first isolated in 1906 by Bordet and Gengou from the sputum of children. Formerly it is called *Hemophilus pertussis.* It is a small ovoid coccobacilli, non motile and non sporing. It is capsulated, but tends to lose the capsule on repeated cultivation. It's bipolar metachromatic granules are demonstrated by Toludine Blue staining. The bacteria are nutritionally fastidious and are usually cultivated on rich media supplemented with blood. On blood agar the organism grows slowly and requires 3-6 days to form pinpoint colonies. The organisms are strict aerobe and grows best at 35-36°C. It doesnot ferment sugars, form indole, reduce nitrates, utilize citrate, hydrolyse urea and catalase, oxidase positive. It is a delicate organism being killed readily by heating, drying and disinfectants. Freshly isolated strains have fimbriae. Organism was sensitive to unsaturated fatty acids, sulfides and peroxides.

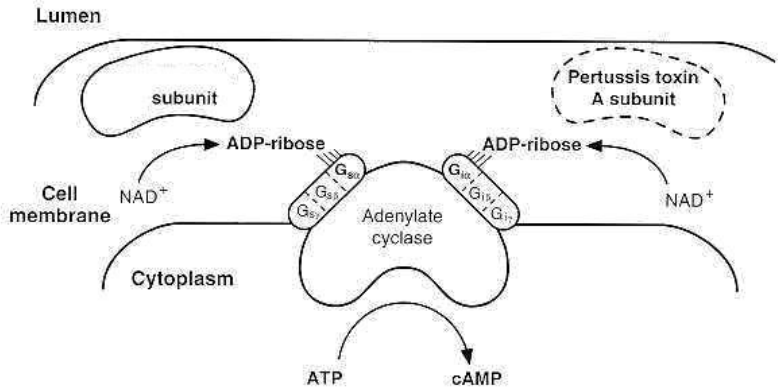
6.3.3. Virulence Factors of B pertussis

Adherence factors and toxins are involved in pathogenicity. These virulent factors are controlled by atleast two sets of gene. The genes may be inactivated at 25°C and activated at 37°C. Adherence mechanisms of *B. pertussis* involve a "**filamentous hemagglutinin**" (FHA), which is a fimbrial-like structure on the bacterial surfaceand **cell-bound pertussis toxin** (**PTx**). Short range effects of soluble toxins play a role as well in invasion during the colonization stage.

6.3.4. Toxins Produced by B. pertussis

B. pertussis produces a variety of substances with toxic activity. It secretes its own **invasive adenylate cyclase**, which enters mammalian cells. This toxin acts locally to reduce phagocytic activity and probably helps the organism to initiate infection. This toxin is a 45 kDa protein that may be cell-associated or released into the environment. It produces a highly **lethal toxin** (formerly called dermonecrotic toxin), which causes inflammation and local necrosis to adjacent sites where *B. pertussis* is located. The lethal toxin is a 102-KDa protein composed of four subunits, two with a MW of 24KDa and two with MW of 30KDa. It causes necrotic skin lesions when low doses are injected subcutaneosly in mice and is lethal in high doses. The role of the toxin in whooping cough is not known. It produces a substance called the **Tracheal cytotoxin**, which is toxic for ciliated respiratory epithelium and which will stop the ciliated cells from beating. It also stimulates release of cytokine IL-1and so causes fever. It also produces the **Pertussis toxin**, **PTx**, a protein that mediates both the colonization and toxemic stages of the disease. PTx is a two component, A+B bacterial exotoxin. The A subunit (S1) is an ADP ribosyl transferase. The B component, composed of five polypeptide subunits (S2 through S5), binds to specific carbohydrates on cell surfaces. The A subunit gains enzymatic activity and transfers the ADP ribosyl moiety of NAD to the membrane-bound regulatory protein Gi that normally inhibits the eukaryotic adenylate cyclase. The Gi protein is

inactivated and cannot perform its normal function to inhibit adenylate cyclase. The conversion of ATP to cyclic AMP cannot be stopped and intracellular levels of cAMP increase. This has the effect to disrupt cellular function and in the case of phagocytes, to decrease their phagocytic activities such as chemotaxis, engulfment, the oxidative burstand bactericidal killing. Systemic effects of the toxin include lymphocytosis and alteration of hormonal activities that are regulated by cAMP, such as increased Insulin production (resulting in hypoglycemia) and increased sensitivity to histamine (resulting in increased capillary permeability, hypotension and shock). This alters both AMI and CMI responses and may explain the high freqency of secondary infections that accompany pertussis



Lipopolysaccharide. As a Gram-negative bacterium *Bordetella pertussis* possesses lipopolysaccharide (endotoxin) in its outer membrane, but its LPS is unusual. It is heterogeneous, with two major forms differing in the phosphate content of the lipid moiety. The alternative form of Lipid A is designated as Lipid X. The unfractionated material elicits the usual effects of LPS (i.e., induction of IL-1, activation of complement, fever, hypotension, etc.).

Agglutinogens - The agglutinogens are surface antigens responsible for agglutination of the bacterial cells in the presence of their corresponding antibodies. To date, 14 different agglutinogens (AGG 1 through AGG 14) have been distinguished. AGG1 is specific for *B pertussis*.

Outer Membrane Proteins - At least four different Outer Membrane Protein structures are distinguished on *B pertussis*; they are designated OMP 15, OMP 18, OMP 69and OMP 91. They are believed to be protective antigens.

6.3.5. Symptoms

Pertussis begins as a mild upper respiratory infection. Symptoms resemble those of a **common cold, runny nose, slight fever and a mild cough**. Within two weeks, the cough becomes more severe and is characterized by rapid coughing followed by a **high pitched whoop.** Thick, clear mucus may be discharged. The incubation period is usually five to 10 days, but may be as long as 21 days. A person can transmit

Pertussis from seven days following exposure to three weeks after the onset of coughing episodes.

6.3.6. Pathogenesis

The agent of whooping cough is transmitted primarily via droplets. Infection results in colonization and rapid multiplication of the bacteria on the mucous membranes of the respiratory tract.

Bordetella pertussis colonizes the cilia of the mammalian respiratory epithelium. Filamentous hemagglutinin is a large (220 KDa) protein that forms filamentous structures on the cell surface. FHA binds to galactose residues on the surface of ciliated cells. One of the toxins of *B. pertussis*, the pertussis toxin (PTx), is also involved in adherence to the tracheal epithelium.

Antibodies against PTx components prevent colonization of ciliated cells by the bacteria and provide effective protection against infection. Thus, pertussis toxin is clearly an important virulence factor in the initial colonization stage of the infection.

The Second or Toxemic stage of Pertussis follows relatively nonspecific symptoms of the colonizaton stage. It begins gradually with prolonged and paroxymal coughing that often ends in a characteristic inspiratory gasp (whoop). During the second stage, *B. pertussis* can rarely be recoveredand antimicrobial agents have no effect on the progress of the disease. This stage is mediated by a variety of soluble toxins. On the basis of symptoms produced by the organisms, the disease stage was divided into three. They are Catarrhal stage, Paroxymal stage and Convolesent stage

Catarrhal stage - It is characterized by low grade fever, rhinorrhoea and progressively worsening cough and also mucous membrane inflammation
Paraximal stage - Patient tries to cough up the mucous by making 5-15 rapidly continuous cough followed by the characteristic whoop-a hurried deep inspiration. the total blood leucocyte levels may resemble those of leukemia ($\geq$ 100,000/mm^3), with 60 to 80 percent being lymphocytes
Convolescent stage - It is the recovery stage

Stages	**Incubation period**	**Catarrhal**	**Paroxymal**	**Convolescent**
Duration	7-10 days	1-2 weeks	2-4 weeks	3-4weeks
Symptoms	None	low grade fever, rhinorrhoea and progressively worsening cough and also mucous membrane inflammation	Rapidly continuous cough followed by the characteristic whoop.	Diminished paroxymal cough, pneumonia
Culturing	None	Possible	Possible	Rare

6.3.7. Laboratory Diagnosis

In the early stages of infection, the organism is present in enormous numbers in the patient's respiratory secretions and is rarely detected in smears. Samples are collected by the following methods.

Cough plate method - Here the culture plate is held about 10-15 cm in front of the patients mouth during induced coughing, so that droplets of respiratory exudate impinge directly on the medium.

Post Nasal Swab - Secretions of the posterior pharyngeal wall are collected with a alginate swab on a bent wire passed through mouth

Pernasal Swab - Here a swab on flexible wire is passed along the floor of the nasal cavity and material collected from pharyngeal wall. This method yields highest percentage of isolation

All specimens should be immediately plated onto Bordet-Gengou medium and is incubated at 35-36°C for 48-72 hours. Colonies are small dome shaped, smooth opaque greyish white, refractile and glistening, resempling mercury drops. A hazy zone of hemolysis surrounds Colonies. For routine use, Charcoal-Blood agar (REGAN-LOWE medium) is most widely used. A (2,6-O-dimethyl)-b-cyclodextrin supplemented STAINER-SCHOLTE broth can be used as an enrichment medium. The *Bordetella* species do not need factors X and V (NAD+ and hemin).

Modern serologic techniques, such as Enzyme-Linked Immuno Sorbent Assay (ELISA), have been used to detect IgG, IgM, IgAand IgE antibodies.

6.3.8. Treatment

Treatment with Erythromycin, which is usually considered the antibiotic of choice, will eliminate viable *B pertussis* organisms from the respiratory tract within a few days.

6.3.9. Control

Several new **acellular vaccines** have been developed from purified components of *B. pertussis.* The Pertussis vaccine has been given in combination with vaccines against Diphtheria and Tetanus. The combination is known as the **DTP** vaccine. Recently, infants have been able to receive a vaccine that combines the **DTP** vaccine with the vaccine against *Haemophilus influenzae* type b meningitis (Hib). This vaccine is called **DTPH**. The Diphtheria-Tetanus-Pertussis vaccine using acellular pertussis is known as **DTaP**. The Diphtheria-Tetanus-Pertussis vaccination is given in five doses: at 2, 4, 6, 12-18 months and 4-6 years of age.

Check Your progress

6.1. Why pertusis is called Whooping cough

6.2. What is fastidious organism

6.3. How can you demonstrate metachoromatic granules in B. pertusis

6.4. YERSINIOSIS.

6.4.1. Introduction

Yersiniosis is an infectious disease caused by two zoonotic bacterium of the genus Yersinia. It is other than Yersinia pestis, which causes plaque. Most yersiniosis infections among humans are caused by Yersinia enterocolitica. Yersinia pseudotuberculosis also causes yersiniosis. Infection with Y. enterocolitica occurs most often in young children. The infection is thought to be contracted through the

consumption of undercooked meat products, unpasteurized milk, or water contaminated by the bacteria.

6.4.2. Causative agent

Yersinia enterocolitica is a gram negative bacteria belongs to class gamma proteobactera, order enterobacterials and family yersiniaceae. It is a coccobacilliand are facultative anaerobe. It is positive to ornithine decarboxylase, sucrose and sorbitol fermentation.

6.4.3. Virulence factors

Adhesions, invasions, antiphagocytic proteins, heat stable entero toxin.

6.4.4. Signs and symptoms

Infection with *Y. enterocolitica* can cause a variety of symptoms. Common symptoms in children are fever, abdominal pain and bloody diarrhea. Incupation period is 4 to 7 days. In adults, right-sided abdominal pain and fever may be the predominant symptoms. Complications are skin rash, joint pains, ileitis, erythema nodosum

6.4.5. Pathogenesis

The organism enters through oral cavity. After entry, Yersinia adhesion A protein (YadA) mediates mucus and epithelial cell attachment and, in concert with invasin, promotes host cell invasion. YadA is a multifunctional, surface-exposed virulence factor encoded on the pYV virulence plasmid that confers the ability to adhere to extracellular matrix proteins. Adhesion protein initiates internalization by binding to extracellular fibronectin, collagen I, IIand IVand laminin, albeit with different affinities thus promoting variable virulence properties. Adhesion protein elicits an inflammatory response in epithelial cells by inducing mitogen-activated protein kinase-(MAPK-) dependent interleukin (IL)-8 production and by contributing to the resulting intestinal inflammatory cascade. Interaction of adhesion protein with collagen has been proposed to contribute to chronic yersiniosis infections, such as the development of reactive arthritis

6.4.6. Lab Diagnosis

Yersiniosis usually is diagnosed by detecting the organism in the stool of an infected person. The organism can also be recovered from other sites, including the throat, lymph nodes, joint fluid, urine, bileand blood. The organism grow effectively on Cefsulodin irgasan Novobiocin (CIN) Agar and produce Bulls eye colony. It also grows in Mac conkey agar and produc e LF Colonies. This organism is Indole negative and urase Positive.

6.4.7.Treatment

The drugs of choice are doxycycline and an aminoglycoside. Alternatives include cefotaxime, fluoroquinolones and co-trimoxazole.

Check Your progress

6.4. Is yersiniosis is due to zonotic bacteria

6.5. Name the media used for Yersinia cultivation

6.6. Mention major complications of Yersiniosis

6.5. LET US SUM UP

Pertussis, or Whooping cough, is a highly contagious disease of respiratory tract. It is caused by *Bordetella pertussis.* It is a small ovoid coccobacilli, non motile and non sporing. It is capsulated, but tends to lose the capsule on repeated cultivation. It's bipolar metachromatic granules are demonstrated by Toludine Blue staining.. *B. pertussis* produces a variety of substances with toxic activity. It secretes its own invasive adenylate cyclase, lethal toxin, Tracheal cytotoxin, Pertussis toxin. Pertussis begins as a mild upper respiratory infection. Symptoms resemble those of a common cold, runny nose, slight fever and a mild cough. *Bordetella pertussis* colonizes the cilia of the mammalian respiratory epithelium. Filamentous hemagglutinin is a large (220 KDa) protein that forms filamentous structures on the cell surface. FHA binds to galactose residues on the surface of ciliated cells. One of the toxins of *B. pertussis*, the pertussis toxin (PTx), is also involved in adherence to the tracheal epithelium. In the early stages of infection, the organism is present in enormous numbers in the patient's respiratory secretions and is rarely detected in smears. Treatment with Erythromycin, which is usually considered the antibiotic of choice, will eliminate viable *B pertussis* organisms from the respiratory tract within a few days.

Yersiniosis is an infectious disease caused by Yersinia enterocolitica. It is due to consumption of undercooked meat products, unpasteurized milk, or water contaminated by the bacteria. Yersinia enterocolitica is a gram negative bacteria belongs to class gamma proteobactera, order enterobacterials and family yersiniaceae. Adhesions, invasions, antiphagocytic proteins, heat stable entero toxin are the virulent factors. . Common symptoms in children are fever, abdominal pain and bloody diarrhea. Incupation period is 4 to 7 days. Complications are skin rash, joint pains, ileitis, erythema nodosum. The organism enters through oral cavity. After entry, Yersinia adhesion A protein (YadA) mediates mucus and epithelial cell attachment and in concert with invasin, promotes host cell invasionInteraction of YadA with collagen has been proposed to contribute to chronic yersiniosis infections, such as the development of reactive arthritis. Yersiniosis usually is diagnosed by detecting the organism in the stool of an infected person. The organism grow effectively on Cefsulodin irgasan Novobiocin (CIN) Agar and produce Bulls eye colony. The drugs of choice are doxycycline and an aminoglycoside.

6.6. UNIT END EXERCISES

Two Mark Questions

Who is Bordet and Gongou

What are the modes of action of pertusis toxin?

Mention virulent properties of Boretella pertusis.

Define Pertusis
What id DPT
Mention characters of *Yersinia enterocolitica*
Listout symptoms of *Yersinia enterocolitica*

Five Mark Questions

Explain Pertusis toxin
Give general characters of Boretella pertusis.
Explain symptoms of pertusis
Explain pathogenesis of Pertusis
How can you identify Boretella pertusis
Describe pathogenesis of Yersiniosis
Explain diagnosis and Treatment of yersiniosis

Ten Mark Questions

Write an essay on Pertusis.
Explain various stages of pertusis infection
Write a detailed note on Yersiniosis

6.7. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

6.1. Pertusis is a intensive cough. It ends with hurried deep inspiration and whoop sound. Hence this is called whooping cough.
6.2. Those organisms require special nutrients for growth are called fastidious microorganism.
6.3. Using Toludine blue staining
6.4. Yes, it is maninly transmitted through animal products.
6.5. CIN Medium
6.6. Skin rash, Joint pain, erythema nodosum.

6.8. SUGGESTED READINGS

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NOTES

UNIT VII ENTERIC GRAM NEGATIVE BACILLI AFB CELL WALL LESS BACTERIA

Summary

7.1 INTRODUCTION

Enteric gram negative bacteria are belongs to the Enterobacteriaceae. It is a a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera (Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteusand others). The Enterobacteriaceae are facultative anaerobes or aerobes, ferment a wide range of carbohydrates, possess a complex antigenic structureand produce a variety of toxins and other virulence factors. Acid-fast bacteria include the mycobacteria, of which there are more than 30 well-characterized members of the genus. These bacteria are gram-positive, aerobic, non-sporulating, non-motileand often pleomorphic. They are typically smaller than other bacteria. Mycobacteria are acid-fast because of lipid-rich cell envelope. Cell wall–deficient bacteria (CWDB), also known as L-phase or L-formbacteria, are bacterial variants that lack a cell wall, although they may in fact possess small amounts of peptidoglycan. The name L-formwas given to these bacteria because they were discovered at the Lister Institute in London. L-form bacteria are distinct from mycoplasmas, because Mycoplasma spp. do not originate from bacteria that normally possess a cell wall.

7.2 OBJECTIVES

After learning this chapter reader will be able to

Distingusish gram negative bacteria, acid fast bacteria and cell wall less bacteria.

Understand pathogenic properties of enteric bacteriam, Mycobacteria and Mycoplasma

Understand diagnosis of cholera, salmonellosis, tuberculosis and pneumonia.

Understsnd the process of treatment.

7.3. VIBRIOSIS

7.3.1. Introduction

Disease caused by Vibrio are called Vibriosis. **Cholera** is a severe diarrhoeal disease caused by the bacterium under the family Vibrionaceae. It is an acute illness. The infection is often mild or without symptoms, but sometime it can be severe disease characterized by profuse watery diarrhoea, vomiting, leg cramps and resulting in acidosis and hypervolumic shock.

7.3.2. Causative Agent

Vibrio cholerae is a causative agent of cholera. It is a Gram-Negative Comma shaped bacilli with single polar flagellum. It was first isolated in pure culture by Robert Koch in 1883.Most Vibrios have relatively simple growth factor requirements and will grow in synthetic media with glucose as a sole source of carbon and energy. However, since Vibrios are typically marine organisms, most species require large amount of NaCl or seawater base for optimal growth. In liquid media Vibrios are motile by polar flagella that are enclosed in a sheath continuous with the outer membrane of the cell wall. Generation for *Vibrio cholerae* is less than 30 minutes.

Biochemical Characters

Indole	–	Positive
Methyl red	–	Positive
VP	–	Positive
Citrate	–	Positive
TSI	–	K/A : no gas : no H2S .
Motile		
Urease	–	Negative
Catalase	–	Positive
Oxidase	–	Positive
Phenyl alanine	–	Negative
Lysine decarboxylase	–	Positive
Arginine	–	Positive
Ornithine	–	Positive
ONPG	–	Positive
Nitrate	–	Positive

7.3.3 Antigenic types

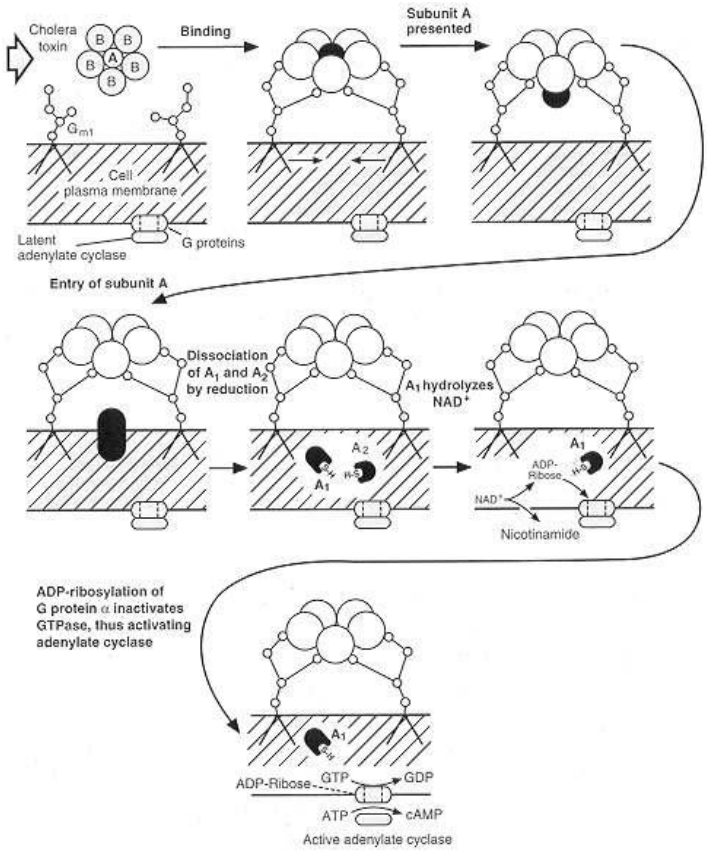
Vibrio that cause epidemic infection have been subdivided into two biotypes: Classic and EI tor. Both biotypes (EI tor and Classic) contain two major serotypes, Inaba and Ogawa. These serotypes are differentiated in agglutination and vibriocidal antibody tests on the basis of their dominant heat stable lipopolysacchride somatic antigens.

7.3.4. Pathogenesis

Cholera is a serious epidemic disease that has killed millions of people and continues to be a major health problem worldwide. Cholera is acquired by drinking water that has been contaminated with human feces or by eating food that has been washed in contaminated water. *Vibrio cholerae*, persists in the environment because it can grow in salt water or in freshwater.

Adhesions, Neuraminidase, Motility, Chemotaxis and Toxin Production are the important virulent factors of *Vibrio cholerae*.

It also have the capability to survive the gastric secretions and low pH of the stomach, they are well adapted to survival in the small intestine. *V. cholerae* is resistant to bile salts and can penetrate the mucous layer of the small intestine, possibly aided by secretion of neuraminidase and proteases. They withstand propulsive gut motility by their own swimming ability and chemotaxis directed against the gut mucosa. Toxin Coregulated Pili (**TCP Pili)** mediates attachment to the intestinal mucosa. Two other possible adhesions in V. cholerae are a surface protein that agglutinates Red Blood Cells (Hemagglutinin) and a group of outermembrane proteins, which are products of the ACF (Accessory Colonization Factor) genes. Nonfimbrial adhesions mediate a tighter binding to host cells .

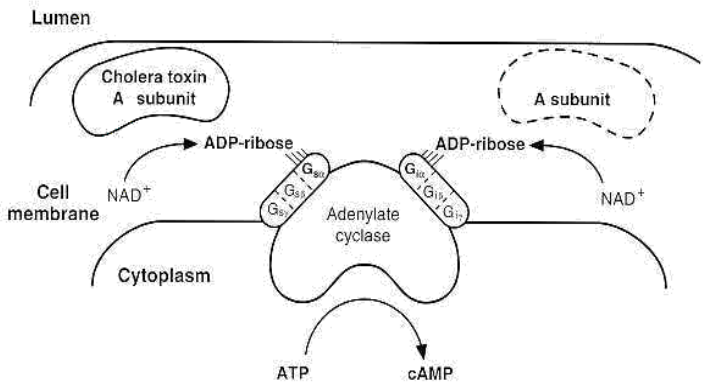


After attachment Cholera bacilli colonize on mucosal surface and produce an Enterotoxin, Cholera toxin that is responsible for Cholera symptoms. Cholera toxin **activates the adenylate cyclase enzyme in cells of the intestinal mucosa** leading to increased levels of intracellular cAMPand the secretion of H_20, Na^+, K^+,$Cl^-$and HCO_3^- into the lumen of the small intestine. The toxin contains **5 binding (B) subunits** of 11,500 daltons, an active **(A1) subunit** of 23,500 daltonsand a **bridging piece (A2)** of 5,500 daltons that links A1 to the 5B subunits. *V. cholerae* Enterotoxin is a product of *ctx* genes. *ctx*A encodes the A subunit of the toxinand *ctx*B encodes the B subunit.The genes are part of the same operon. The transcript (mRNA) of the *ctx o*peron has two ribosome binding sites. The components of cholera toxin is synthesized in the cytoplasm and are assembled in the periplasm after translation. Extra B subunits can be excreted by the cell, but A must be attached to 5B in order to exit the cell A1 and A2 subunits are linked by a disulfide bond. After release of cholera toxin into the lumen, it bound to the oligosaccaride moiety of GM1 gangliocide receptor on host cells'. A1 subunit is released from the toxin, presumably by reduction of the disulfide bond that links it to A2and enters the cell by an unknown

translocation mechanism. One hypothesis is that the 5 B subunits form a pore in the host cell membrane through which the A1 unit passes.

Once fragment A1 entered inside of the cell, it enzymatically catalyzes the transfer of the ADP-ribosylmoiety of NAD to a component of the adenylate cyclase system. The process is complex. Adenylate cyclase (AC) is activated normally by a regulatory protein (GS) and GTP. Hydroxylation of GTP by regulatory protein (G1) leads to inactivation of adenylate cyclase enzyme. In the case of Cholera, A1 fragment catalyzes the attachment of ADP-Ribose (ADPR) to the regulatory protein forming Gs-ADPR from which GTP cannot be hydrolyzed. This leads to the activation of the Adenylate Cyclase.

The net effect of the toxin is to cause cAMP to be produced at an abnormally high rate, which stimulates mucosal cells to pump large amounts of Cl^- into the intestinal contents. H_2O, $Na^+$and other electrolytes flow due to the osmotic and electrical gradients caused by the loss of Cl^-. The lost H_2O and electrolytes in mucosal cells are replaced from the blood. Thus, the toxin-damaged cells become pumps for water and electrolytes causing the diarrhoea, loss of electrolytes,and dehydration that are characteristic of cholera.



7.3.5. Symptoms

The clinical description of Cholera begins after an incubation period of 6-48 hours with sudden onset of massive diarrhoea. The patient may lose gallons of protein-free fluid and associated electrolytes, bicarbonates and ions within a day or two. This loss of fluid leads to, acidosis and shock. The watery diarrhoea is also called "**rice-water stool**" and contains enormous numbers of vibrios. The loss of Potassium ions may result in cardiac complications and circulatory failure. Untreated cholera frequently results in high (50-60%) mortality rates.

7.3.6. Lab diagnosis

Culturing

Specimen : Stool

Transport medium : Alkaline peptone water and Ramakrishna Venkatraman medium

Selective medium : Tri Sodium Citrate Bilesalt Sucrose agar(TCBS)

Colony morphology on TCBS : Circular yellow color colonies

7.3.7. Treatment

Treatment of cholera involves the rapid intravenous replacement of the lost fluid and ions. Following this replacement, administration of isotonic maintenance solution should continue until the diarrhoea ceases. If glucose is added to the maintenance solution it may be administered orally, thereby eliminating the need for sterility and intravenous. administration . Most antibiotics and chemotherapeutic agents have no value in cholera therapy, although a few (e.g. tetracyclines) may shorten the duration of diarrhoea and reduce fluid loss.

Check Your Progress

7.1. What is the other name for Vibriosis

7.2. What is Rice water stool

7.3. Name transport medium for Vibrio

.

7.4. SALMONELLOSIS.

7.4.1. Introduction

Infection caused by Salmonella are commonly called Salmonellosis. Enteric fever is caused by Salmonnella. Enteric fever is a collective term used for invasive infections caused by a small group of bacteria called Salmonella. The term invasive is used to describe a tendency to spread, penetrate and intrude. Enteric fever is an important public health problem worldwide. An estimated 14 -27 lakhs people are affected by entric fever in India every year. It is prevalent all over world. Louis gave the name typhoid. Budd pointed out that the disease transmitted through the excreta of patients. Typhoid fevers occurs only in humans.

7.4.2. Causative Agent

Typhoid fever is caused by *Salmonella typhi, Salmonella paratyphi A,Salmonella paratyphi B and Salmonella paratyphi C. Diarrhoe and dysentery are caused by Salmonela enterocolitica*

General characters of salmonella are Gram negative, Straight rods, Motile by peritrichous flagella, Facultative anaerobes, Respiratory and fermentative type metabolism, Oxidase negative, Catalase positive, Some strains produce hydrogen sulfide, Non lactose fermentor, Indole negative, Sucrose negative, Mannitol positive, Malanonate positive, Glucose positive, Citrate utilization positive, Glucose positive

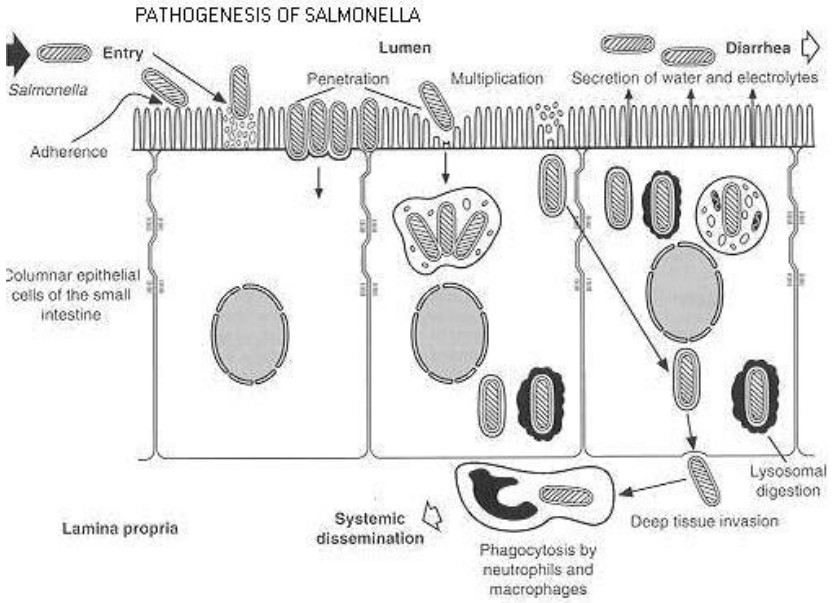
Cultural Characters of Salmonella are Salmonella grow on ordinary media, On MacConkey agar it produce small, circular, translucent, NLF colonies. In Wilson Blair Bismuth Sulfite Medium, the colonies are jet black with metallic sheen colonies. On Salmonella-Shigella agar black centered colonies were observed. On XLD agar slight pink colonies with black centered were observed. On Deoxycholate Citrate agar black colonies were developed

7.4.3. Virulent factors

Endotoxin
Invasions

Catalase,
Superoxide dismutase

Cationic proteins
Factors involved in resistance to acidic pH:
Vi factor
Aromatic aminoacid synthesizing factors.



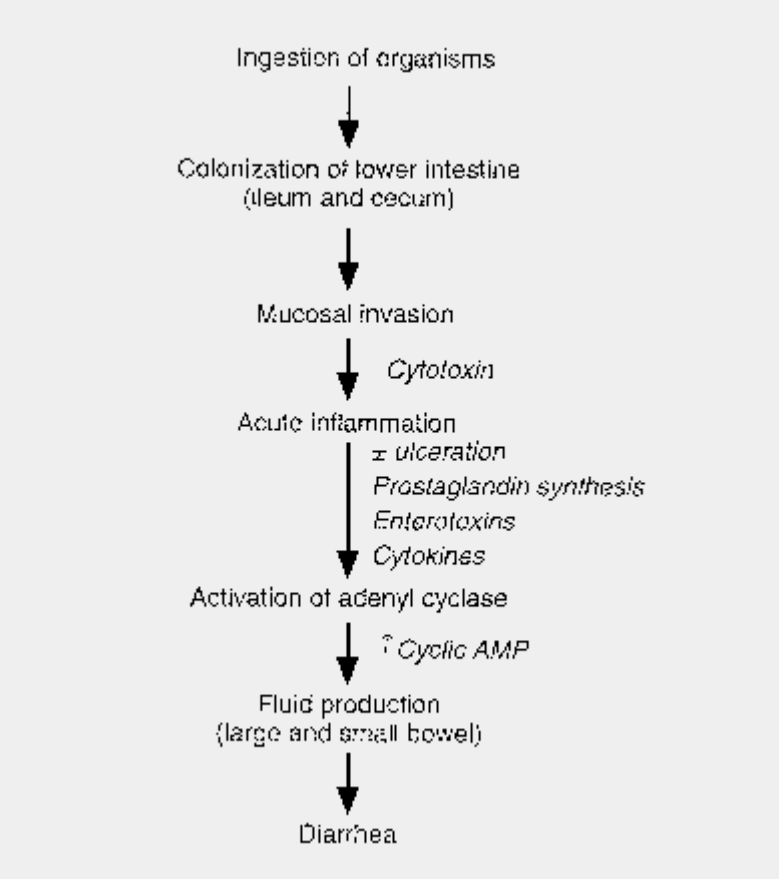
7.4.4. Symptoms

Fever,
Rash,
Relatively lower pulse rate
Coated tongue,
Diffuse tenderness in the abdomen,
Enlarged liver and spleen,

Head ache,
Pain in the abdomen,
Vomitting,
Diarrhoea,
Bleeding from the nose,
Dry cough.

7.4.5. Pathogenesis

Salmonellosis is transmitted by fecal oral route through contaminated food and water. The ingested organisms are mostly destroyed in the stomach. Sufficient number of bacilli passes through the gastric acid barrier and reaches the duodenum, where they multiply in alkaline medium. In the small intestine, the bacilli attack themselves on the surface of epithelial cells of the villi and pass through them to the submucosal coat, where they are phagocytosed by Neutrophils and Macrophages. The virulent bacilli resist intracellular killing and multiply within these cells. These cells enter the Mesenteric lymphnodes, where after a period of multiplication, the bacilli invade the blood stream and primary bacteremia follows. Cells rapidly clear blood stream by Mononuclear Phagocytic System in the liver, bone marrow, spleen, lung and lymphnodes. Thus the internal organs are infected during primary bacteremia in first 7-10 days.



After primary bacteremia organisms enters into the Intestinal Lymphnodes. During 10th day of infection parasitized cells undergo necrosis and the bacilli pass into the blood leading to secondary and heavier bacteremia, which corresponds with the onset of clinical illness at about 14th day after ingestion. During this period some organisms undergo lysis, thereby liberating endotoxin in the circulation. The bacteremia and toxemia cause pyrexia and other symptoms.

From blood stream some organisms localize in organs eg. Gallbladder, Liver, Spleen etc. Some organisms are discharged from the gallbladder into the intestine which cause the inflammation of payers patches of intestine produces necrosis and sloughing of the infected follicles with resultant typhoid ulcers which may lead to hemorrhage and perforation.

The febrile illness often causes severe Mental cloudiness and Headache. Skin rashes known as Rose Spot may appears on chest and abdomen in second and third week. The organism appear in stool during second and third week and in during third to fourth week.

Normally duration of infection varies from 7-14 days in Typhoid fever. 4-5 days for Paratyphoid fever. Typhoid fever is severe than Paratyphoid fever.

7.4.6. Complications

Bleeding from intestine, Perforation of the intestine, Enteric encephalopathy, Kidney failure, Meningitis, Osteomyeletis, Loss of weight, Mental confusion.

7.4.7. Epidemiology

Salmonella typhi maintained in nature by human carriers. There are three main types of typhoid carriers, Convolescent, temporary and chronic. One of the notorious carriers is Typhoid Mary young Irish cook living in Newyark City in early 1900s. She is responsible for 53 cases of typhoid during a 15- year period. Mostly transmitted through fecal oral route. Human is the only natural host. It can occur at any time during the year but common during July - September.

7.4.8. Laboratory Diagnosis

Collection of specimens - Blood (or bone marrow or clot), Stool and Urine are the clinical samples

Isolation of the organism

Blood culture

The organisms can be best isolated during the first 7-10 days of the illness, but in at least half the cases the organisms can still be isolated in the second and the third weeks. Add a minimum of 10 ml of venous blood from an adult patient to each of 50 ml of sodium taurocholate broth and glucose broth. From children at least 5 ml blood should be inoculated in each of the bottles. Incubate the inoculated media at 37^{o}C overnight and subculture onto MacConkey agar and Blood agar. Extend the incubation of blood culture bottle upto 7 days if subcultures fail to yield any bacterial growth. Subculture again on day 7 and if no growth is obtained blood culture may be declared as negative for Enteric fever bacilli.Periodic subcultures are made after day 2, 5 and 7 on MacConkey agar. No growth after seven days may be regarded as negative. Very rarely organisms may appear as colonies after 30 days of incubation.

Culture of faeces

In at least 50% of the cases culture of the feces is positive in first week, so fecal culture should be attempted in the first week of illness itself. The isolation of *S.typhi* greatly increases in second or third week.

Rectal swabs give inferior results than fecal culture. Culture on Deoxycholate Citrate Agar (DCA) and Wilson and Blair Bismuth Sulphite Brilliant Green Agar using Selenite F as enrichment medium. XLD agar gives better results in isolating Salmonella from feces. It is recommended to use two enrichment media and two selective media to improve the isolation rate.

Urine culture

Urine culture is positive in a quarter to one third of cases, but there seems to be no regular excretion pattern in the urine. Bacteriuria is usually limited to the first few weeks of the illness and in great majority of the patients. The urine is free from organisms weeks before the feces becomes negative.

Serodiagnosis
Detection of antibodies

A large number of serological tests have been devised to detect and titrate the antibodies against common agents of Enteric Fever. The tube agglutination test (Widal test) has great historical but little diagnostic importance unless four fold rise is exhibited in two samples drawn at interval of 10-14 days from the start of the illness.

WIDAL TEST - In this test the patient's serum is tested by tube agglutination test for its titres of antibodies against H,O and Vi suspensions of enteric fever bacteria.

7.4.9. Prevention

Sanitary measures and vaccines are the two main ways to prevent Enteric Fever. Key sanitary measures include :
Provision of safe drinking water
Safe disposal of human sewage
Maintaining high standards of food hygiene
Care in detecting, monitoring and treating chronic carriers
Individual protection measures includes :
Drinking boiled, iodinated or chlorinated water
To be careful with food prepared from outside of house
To take typhoid vaccine
Avoiding eating of raw or partially cooked eggs
Washing hands with soap and water after going to toilet and before preparing or handling food
People who have recovered from fever need to use separate towels till they get completely cured.

7.4.10. Treatment

Chloramphenicol
Ampicillin
Amoxicillin
Cotrimoxazole
Ciprofloxacin
Ofloxacin
Norfloxacin
Pefloxocin
Ceftriaxone
Cefaperazone
Cofotaxine

Check Your Progress

7.4. What is the grams nature of Salmonella.

7.5. Mention the type of flagella present in Salmonella.

7.6. name of samples used for the recovery of Salmonella

7.5. TUBERCULOSIS

7.5.1.Introduction

Tuberculosis is one of the lower respiratory tract infection. It is a progressive granulomatous disease of the lungs. The members of the genus Mycobacterium cause it. It is a Acid-Fast Bacilli. It is an ancient disease, recognized in skeleton from the Stone Age and in bones from

some of the early Egyptian Mummies. Although the infectious nature of Tuberculosis was established by Villemin arroun (1865). The causative agent was first described by Robert Koch during the year 1882 and named as **Mammalian Tubercle Bacilli**. It is caused by very closely related species of Mycobacterium that are *Mycobacterium tuberculosis* (Human) and *Mycobacterium bovis* (Animals)

7.5.2. Causative Agent

It is a Acid-Fast Bacilli. It is due to Mycolic acid content of the cell wall. It is fairly large Nonmotile rod shaped, nonspore forming, non capsulated bacterium. In tissue it is thin straight rods, occurring singly, in pairs or in clumps. It is Weekly Gram positive, Obligate Aerobes, Slow grower and generation time is 6-12 hours. Optimum temperature is 37°C, pH is 6.4 –7

They only grown in the media containing egg, asparagine,potatoes and serum.

7.5.3. Factors responsible for pathogenesis

Mycobacterium has large quantities of lipids. It includes Mycolic acids (long chain fatty acid C78-C90) waxes and phosphatides. Muramyl dipeptide complexed with mycolic acid can cause granuloma formation. Phosphatide induce Tubercle formation. Lipid can cause accumulation of macrophages and neutrophiles.

Virulent strains of tubercle bacilli form microscopic **Serpendine cords** in which bacilli are arranged in parellel chains. Cord formation is correlated with virulence. A cord factor (Trehalose 6,6 dimycolate) inhibits migration of leukocytes, causes chronic granuloma. Polysaccarides induce hypersensitivity reaction. Protein found along with wax induces antibody formation.

Uses of high concentration of lipid in *M.tuberculosis* are Impermeable to stains and dye. Resistance to many antibiotics. Resistance to killing by acidic and alkaline compounds. Resistance to osmotic lysis via complement deposition. Resistance to lethal oxidation and survival inside the macrophages.

7.5.4. Symptoms

Clinical signs and symptoms develop in only a small proportion (5-10 percent) of infected healthy people. These patients usually present with pulmonary disease; prominent symptoms are Chronic productive cough, Low-grade fever, Night sweats, Easy fatigability and Weight loss. Tuberculosis may present with or also exhibit extrapulmonary manifestations including lymphadenitis; kidney, bone, or joint involvement; meningitis; or disseminated (miliary) disease.

7.5.5. Pathogenesis

M tuberculosis infections occur by airborne transmission of droplet nuclei containing a few viable cells (no morethan three), virulent organisms produced by a sputum-positive individual. Coughing generates

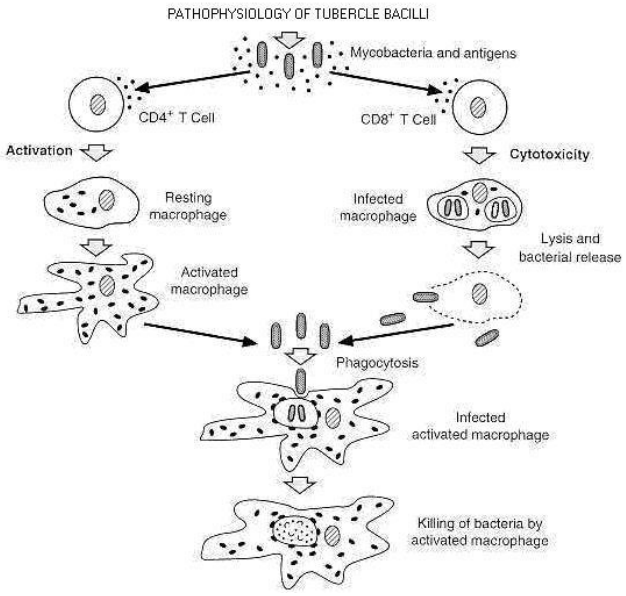
about 3000 droplet nuclei, talking for 5 minutes generates 3000 droplet nuclei but singing generates 3000 droplet nuclei per minute.

After the inhalation of droplet nuclei, the bacilli are deposited in the alveolar spaces of the lungs, where they are non specifically engulfed by alveolar macrophages. However the macrophages are not activated and are unable to destroy the intracellular organisms.

A portion of the infectious inoculum resists intracellular destruction and persists, eventually multiplying and killing the macrophage. Other macrophages also begin to extravasate from peripheral blood. These macrophages also phagocytose the *M tuberculosis* but they are unactivated and hence cannot destroy *M tuberculosis*.

At these stage lymphocytes begins to infiltrate. The lymphocytes , specifically T cells, recognize the antigen , processed and presented through MHC molecule. This results in T cell activation and release of cytokines and other factors.

CD4 cell produce gamma interferon that activate macrophages and enhanced mycobactericidal capabilities of CD4 cells. These cells can limit the replication of intracellular *M tuberculosis* and may kill the bacilli.



CD8 cells attack infected macrophages expressing Mycobacterial antigens and lyse the cells by releasing them from the protective niche and exposing them to activated macrophages.

Activation of macrophages can results in bacterial killing while cytotoxicity may release bacteria form phagocytes and allow their engulfment and destruction by activated macrophages.

TH1 cells produce IL-2 & gamma interferon, which promote the inflammatory reactions and CMI. TH2 cells produce IL-4, IL-5 and IL-10 and promote antibody production Cord factor may be directly cytotoxic to macrophages. Most of the tissue destruction associated with tuberculosis results from cell-mediated hypersensitivity.

Accumulation of Mycobacteria stimulate an inflammatory focus which matures into a granulomatous lesion characterized by a mononuclear cell infiltrate surrounding a core of degenerating epithelioid and multinucleated giant (Langhans) cells. This lesion (called a tubercle) may become enveloped by fibroblastsand its center often progresses to **Caseous necrosis**. Liquefaction of the caseous material and erosion of the tubercle into an adjacent airway may result in cavitation and the release of massive numbers of bacilli into the sputum. In the resistant host, the tubercle eventually becomes calcified.

Early in infection, Mycobacteria may spread distally either indirectly through the lymphatics to the hilar or mediastinal lymph nodes via the thoracic duct into the blood stream, or directly into the circulation by erosion of the developing tubercle into a pulmonary vessel. This is called **Disseminated tuberculosis**. Extrapulmonary hematogenous dissemination results in the seeding of other organs (e.g., spleen, liverand kidneys) and, eventually, reinoculation of the lungs. This was often considered as **Reactivated tuberculosis**.

Miliary lesions, which are small granulomas, resemble millet seeds spread throughout the lung fields known as **Miliary tuberculosis**. Secondary lesions caused by miliary tuberculosis can occur almost any anatomical location but usually involve the genitourinary system, joints lymphnodes. It is two types.

1) Exudative lesion- results from the accumulation of PMNs around *M tuberculosis*. It results in **Soft tubercle.**
2) Productive lesion (granulomatous) it occurs when host become hypersensitive to tuberculoprotein. This situation gives rise to the formation of **Hard tubercle**.

Usually host will begin to control the infection at some point, when primary lesion heals, it becomes fibrous and calcifies. When this happens the lesions referred to as Ghon complex. If the complex contain viable cells these foci are reffered to as Simon foci.

7.5.6. Epidemiology

Tuberculosis is particularly common in groups such as the elderly, the chronically malnourished, alcoholicsand the poor. The prevalence of clinical tuberculosis among the homeless in the United States may be up to 300 times higher than the national average rate. In recent years, the incidence of disease in racial minorities in the United States has been more than five times that observed in whites.

7.5.7. Lab Diagnosis

Infection in an asymptomatic individual can be diagnosed with the help of the intradermal PPD skin test. Intradermal introduction of PPD into a previously infected, hypersensitive person results in the delayed (48-72 hr) appearance of an indurated (raised, hard) reaction with or without erythema. The Mantoux test requires the intradermal injection of a measured volume (0.1 ml) containing a specified quantity

(5 tuberculin units) of PPD. The transverse diameter of induration is measured 48 to 72 hours later. Interpretation varies.

Sample Collection - Sputum should be collected during early morning. Commercial chemiluminescent DNA probes, Gas-Liquid Chromatography, High-Performance Liquid Chromatographyand Thin-Layer Chromatography allow identification of a few species of Mycobacteria within hours after sufficient growth is present on solid or in a liquid medium.

In the future, Nucleic Acid Amplification methods may prove useful for detection of Mycobacteria directly in clinical material within 24 hours or less of specimen receipt. Currently, standardized guidelines for susceptibility testing of mycobacteria have been developed only for isolates of *M tuberculosis* a positive BACTEC TB vial (indirect test), or on sputum specimens that are smear-positive (direct test). Using a broth system, results are available 5-7 days after bottles are inoculated.

LJ medium is used for the cultivation of Mycobacterium tuberculosis.

7.5.8. Treatment and Control

First-line anti-mycobacterial drugs are Isoniazid, Rifampin, Pyrazinamideand Ethambutol. BCG Vaccine is given for prevention.

Check Your Progress

7.7. What is mammalian tubercle bacilli

7.8. What is serpentine cord

7.9. What is military tuberculosis

7.10. What is Ghon complex

7.6. LEPROSY

7.6.1. Introduction

Leprosy is a chronic granulomatous disease of man involving primarily the skin, peripheral nerves and nasal mucosa but capable of affecting any tissue or organs. As per WHO it is defined as a hypopigmented or reddish skin lesion with defnite loss of sensation. It is one of the Nervous system infections. It is a disease of great antiquity, having been recognized from Vedic times in India and from biblical times in Middle East. It probably originates in trophies and spread to rest of the world. Leprosy is also called **Hanson's** disease because Hanson first observed Lepra bacilli in 1868. The first successful propagation of the leprosy bacillus in the laboratory did not occur until 1960. In 1970 it was found that *M.leprae* causes a systemic infection in the nine-banded armadillo.

7.6.2. Causative agent

M. leprae causes leprosy. It was the first bacilli isolated from human. It is one of the least understood bacterium. Hanson, a Norwagion physician isolated *M. leprae* from lepra cells of human beings. It doesnot grows on artificial media. It reaches to the

environment form the nose and upper respiratory tract of persons with leprosy. It is a straight or slightly curved rod. Organisms are found singly or in large masses termed globi. Large numbers of bacilli may be packed in the cells in an arrangement that suggests packets of cigars. It is one of the Acid-Fast Bacilli.

It grows well on footpads of ninebanded armadillo at 30°C. This temperature is obtained by controlling air temperatures at 20°C-25°C.

7.6.3. Pathogenesis

Organism enters to the human body through respiratory route or through skin. Some author says transmission of lepra bacilli requires close contact with infected patients. It mainly attacks nerve cells and grows very slowly in mononuclear macrophages especially the histiocytes of skin and Schwann cells of the nerves. Rate of infection depends on the status of human immune system. Attack of immune cells against affected nerve cells produces nerve damage leading to deformity. On the basis of immunological findings, histopathology and clinical findings Ridley and Jopling have established a classification scheme consisting of five terms of leprosy.

S . No	**Type of disease**	**Immune response**	**Bacilli in skin**	**Bacilli in nasal mucosa**
1	Tuberculoid (TT)	Good	None	None
2	Boderline Tuberculoid (BT)	Less good	None or few	None
3	Boderline (BB)	Partial	Few	None
4	Borderline lepromatous (BL)	Poor	Moderate	Few
5	Lepromatous (LL)	Nil	Many with globi	Many with globi

Among these two forms are stable, they are Tuberculoid and Lepromatous.

TUBERCULOID - In this, skin biopsy specimens show mature granuloma formation in the dermis that consists of epitheloid cells, giant cells and marked infiltration of lymphocytes. Acid Fast Bacilli usually cannot be demonstrated. The organisms invade the nerves and selectively colonize the schwann cells. The larger nerves are swollen and destroyed by granulomas or inflammatory cells. The nerve damage is nonspecific and arises as a consequence of the cellmediated immune response.

LEPROMATOUS - In this there is no cellular immune response. The lesions are small and many. They are shiny with no loss of feeling. Skin and Nasal smears contain many bacteria. 10^9bacilli are observed per gram of tissue. Organisms tend to invade vascular channels, which results in a continuous bacteremia and consistent involvement of the Reticulo Endothelial System. The nerves are also infected but are less than tuberculoid type.

BORDERLINE -The lesions clinically resemble Tuberculoid leprosy but bacteriologically and immunologically resemble lepromatous type. Immunological response ranges from lessgood to poor.

Other terms used in the classification

According to World Health Organization report 1982. Leprosy is divided into two groups, **Paucibacillary** (lesion contains few bacteria) which include all cases of tuberculoid types and some cases of borderline type (BT, TT). **Multibacillary** (they contains large numbers of bacilli) includes all cases of Lepromatous types and some cases of Borderline (BB, BL and LL).

Common symptoms include progressive nerve damage, chronic skin lesionsand ulcerative lesions of mucous membrane deformed faces, loss of fingers and toes.

7.6.4. Laboratory Diagnosis

Specimens

Skin biopsy

Scrapings from lesions and nasal mucosa

1. Direct slit skin smear

Slit skin smears made from skin scrapings from patches and earlobes and punctured material from nodules and nasal mucosal scrapings are stained by modifies Ziehl-Neelsons method using 4-5% sulphuric acid as a decolorizing agent. Smear shows Acid Fast Bacilli arranged in parallel bundles within macrophage (lepra cells) and this confirms the Lepromatous Leprosy. The living cells stain uniformly but dead cells looks fragmented and irregular.

On the basis of bacteriological index of skin smear, assess the sevearity of infections. Bacteriological index is defined as the number of viable bacilli in a lesion, which is assessed from stained smear by oil immersion lens.

1-10 bacilli in 100 fields	=	1+
1-10 bacilli in 10 fields	=	2+
1-10 bacilli per field	=	3+
10-100bacilli per field	=	4+
100-1000bacilli per field	=	5+

More than 1000 bacilli, clumps and groups per field is 6+

On the basis of Morphological index, assess the effect of antibiotic treatment. It is the percentage of uniformly stained bacilli out of the total bacteria present in tissue.

2. Skin and nerve biopsy

Skin biopsy is collected from active edge of the patches and nerve biopsy from thickened nerve for histological confirmation of tuberculoid leprosy when AFB cannot be demonstrated by direct smear.

3. Animal inoculation

The culture of *M.leprae* on artificial media has not yet been achieved. It is possible, however to produce the growth of the organism in certain animals such as the footpads of mouse, rat and Nine Banded Armadillo.

Large number of bacilli is obtained from Armadillo. This type of cultivation was successfully completed during the year1971. Body temperature of the animal is 30°C that favors the growth of the organism.

4. Indirect tests

Lepromin test - Lepromin is a boiled extract of Lepromatous tissue in isotonic saline. When 0.1 ml of lepromin (contain 40 million dead cells) is injected intradermally to an individual, it produces an area of nodular infiltration at the site of injection in skin, which reaches its maximum size in 3-5 weeks. This is a nonspecific skin test, which can be helpful in classifying leprosy and assessing the future course of the disease. One of its main values is in confirming lepromatous leprosy.

7.6.5. Treatment

Dapsone (4,4 diamino diphenyl sulphone) is an effective monotherapy for all types of leprosy till 1982. As per Indian Journal of Leprosy the following drugs were recommended for leprosy patients.

Rifampicin
Dapsone
Clofazimine
Ethionamide
Ofloxacin
Sparfloxacin
Sparfloxacin
Clarithromycin
Mimocycline
Ofloxacin
Prothionamide
Mimocycline
Clathromycin

Check Your Progress

7.11. What is lepra bacilli.

7.12. What is hansons disease

7.13. What is nine banded armadillo.

7.14. Mention the use of dapsone

7.7. PNEUMONIA - Mycoplasma

7.7.1. Introduction

Pneumonia is a lower respiratory infection caused by different types of organisms. One among the organisms is *Mycoplasma pneumonia*. It is a cell wall less organism. It is resembling Pleuro Pneumonia causative agents and are called Pleuro Pneumonia Like Organisms (PPLO). These organisms are now called Mycoplasmas. Mycoplasmas are the smallest and simplest self-replicating bacteria. The Mycoplasma cell contains a Plasma Membrane, Ribosomesand a genome consisting of a double-stranded circular DNA molecule. The Mycoplasmas have no cell wallsand they are consequently placed in a separate class Mollicutes (mollis, soft; cutis, skin). Mycoplasmas have been nicknamed the "**crabgrass**" of cell cultures because their infections are persistent, frequently difficult to detect and diagnoseand difficult to cure.

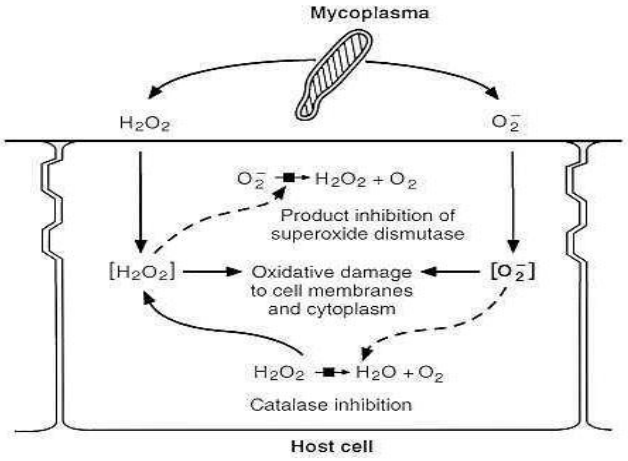
7.7.1. Causative agent

The term atypical pneumonia was coined in the early 1940s to describe pneumonias caused by Mycoplasma. It is isolated by Eaton and

associates and was called *Eaton agent*. This agent was identified as a Mycoplasma *pneumoniae*. It causes subclinical infection, upper respiratory diseaseand bronchopneumonia in humans. Symptoms are remittent fever, coughand headache persist for several weeks. One of the most consistent clinical features is a lung convalescence, which may extend from 4 to 6 weeks. Several unusual complications have been noted, including hemolytic anemia, polyradiculitis, encephalitis, aseptic meningitisand central nervous system illness such as Guillain-Barré syndrome. In addition, pericarditis and pancreatitis have been observed.

7.7.3. Distinguishing Properties of Mycoplasma

The coccus is the basic form of all Mycoplasmas in culture. In most Mycoplasma cultures, elongated or filamentous forms also occur. The filaments tend to produce truly branched mycelioid structures, hence the name Mycoplasma (myces, a fungus; plasma, a form). Mycoplasmas reproduce by binary fission. *Mycoplasma pneumoniae* is a pathogen of the respiratory tract, adhering to the respiratory epithelium, primarily through the attachment organelle. One of the most useful distinguishing features of Mycoplasmas is their peculiar fried-egg colony shape, consisting of a central zone of growth embedded in the agar and a peripheral one on the agar surface. The Mycoplasma genon circular, double stranded slowly, particularly on primary isolation.



Proposed mechanism of oxidative damge to host cells by adhering *M pneumoniae* by increasing concentrations of H_2O_2 and O_2-

7.7.4. Pathogenesis

All Mycoplasmas are parasites of humans, animals, plants, or arthropods. The primary habitats of human and animal Mycoplasmas are the mucous surfaces of the respiratory and urogenital tracts. Most Mycoplasmas that infect humans and other animals are surface parasites, adhering to the epithelial linings of the respiratory or urogenital tracts. Initial interaction between host cell and parasite releases toxic metabolites, which will cause tissue damage. Membrane fusion releases hydrolytic enzymes into the host cell leads to host cell damage. The H_2O_2 and O_2- excreted by the Mycoplasma penetrate into the host cell and cause oxidative damage.

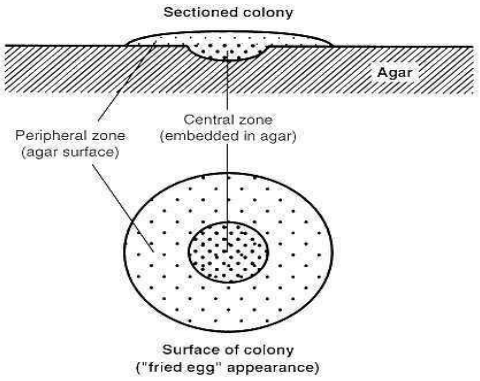
Toxins are rarely found in Mycoplasmas. Hydrogen peroxide (H_2O_2), the end product of respiration in Mycoplasmas, has been implicated as a major pathogenic factor responsible for the lysis of erythrocytes. *M pneumoniae* inhibits host cell catalase by excreting

superoxide radicals (O_2-). Mycoplasmas activate macrophagesand induce cytokine production and lymphocyte proliferation. Thus, in the case of *M pneumoniae*, the host may be largely responsible for the pneumonia by mounting a local immune response to the parasite. *M pneumoniae* can be found in most children 2 to 5 years of age, although the illness occurs with greatest frequency in individuals 5 to 15 years of age.

7.7.5. Epidemiology

Mycoplasma pneumoniae accounts for 8 to 15% of all pneumonias in young school-age children. In older children and in young adults, the organism is responsible for approximately 15 to 50 percent of all pneumonias. *Mycoplasma pneumoniae* appears to require close personal contact to spread; successful spreading usually occurs in families, schoolsand institutions. The incubation period ranges from 2 to 3 weeks.

7.7.6. Lab Diagnosis



Culture is essential for definitive diagnosis. A routine mycoplasma medium consists of heart infusion, peptone, yeast extract, salts, glucose or arginineand horse serum (5 to 20%). Fetal or newborn calf serum is preferable to horse serum. To prevent the overgrowth of the fast-growing bacteria that usually accompany Mycoplasmas in clinical materials, Penicillin, Thallium acetate or both are added as selective agents. For *M pneumoniae* isolation, nasopharyngeal secretions are inoculated into a selective diphasic medium (pH 7.8) made of Mycoplasma broth and agar and supplemented with glucose and phenol red. When *M pneumoniae* grows in this medium, it produces acid, causing the color of the medium to change from purple to yellow. Broth from the diphasic medium is subcultured to Mycoplasma agar when a color change occurs, or at weekly intervals for a minimum of 8 weeks. One of the most useful distinguishing features of Mycoplasmas is their peculiar fried-egg colony shape, consisting of a central zone of growth embedded in the agar and a peripheral one on the agar surface.

7.7.7. Treatment

The Mycoplasmas are sensitive to Tetracyclines, Macrolidesand the newer Quinolones. Tetracycline or Erythromycin is recommended for treatment of *M pneumoniae* pneumonia.

Check Your Progress

7.15. What is PPLO

7.16. Is Penicillin is used for the treatment of Mycoplasma infections.

7.17.Is Mycoplama activate macrophages

NOTES

7.8. LEPTOSPIROSIS.

7.8.1. Introduction

Leptospirosis is a relatively rare bacterial infection that affects people and animals. It can pass from animals to humans when an unhealed break in the skin comes in contact with water or soil where animal urine is present. Several species of the Leptospira genus of bacteria cause leptospirosis. It can progress to conditions such as Weil's disease or meningitis, which can be fatal.

7.8.2. Causative agent

Leptospira interogans is the species responsible for this disease. It is a spirochaete. It is a very thin, actively motile, oblicately aerobic, helical rod shaped bacterium. It is a spiral organism with 5-15μm long and 0.1μm wide. There are three important serogroups in *Leptospira interogans.* They are *L. icterohaemorrhagiae, L. conicola and L. hebdomadis.* These thre serogroups are responsible for human leptospirosis.

7.8.3. Transmission

The condition does not usually pass from one person to another. The bacteria can enter the body through open wounds, the eyes, or mucous membranes. Animals that transmit the infection to humans include rats, skunks, opossums, foxesand raccoons. Leptospirosis is more common in tropical areas. Leptospirosis is a bacterial infection.

7.8.4. Symptoms

Incubation period is 1-2 weeks. High fever, Headache, Chills, Muscle aches, Vomiting, Jaundice (yellow skin and eyes), Red eyes, abdominal pain, Diarrhea, Rash. Leptospira causes hepatorenal damage which is called weils diseases, naming after researcher who identified *icterohaemorrhagic* fever.

7.8.5. Pathogenesis

Leptospira enters the body through breaches in the skin or through the mucous membrane, then into the bloodstream. The bacteria later attach to the endothelial cells of the blood vessels and extracellular matrix . The flagella of the bacteria help it move between cell layers. Endothelial cells of the capillaries are then activated by the presence of these bacteria. The endothelial cells produce cytokines and antimicrobial peptides against the bacteria. These products regulate the coagulation cascade and movements of white blood cells. Leptospira binds to a variety of cells such as fibroblasts, macrophages, endothelial cellsand kidney epithelial cells. Leptospira is well adapted to oxidative environment. Surface leptospiral immunoglobulin-like (Lig) proteins such as LigB, whose gene is found in all pathogenic Leptospira species, help Leptospira bind to several host proteins such as complement proteins, thrombin, fibrinogenand plasminogen.

In the bloodstream, Leptospira can activate host plasminogen to become plasmin that breaks down extracellular matrix (ECM), degrades

fibrin clots and complemental proteins (C3b and C5) to avoid opsonisation. The bacteria can also recruit complement regulators such as Factor H, C4b-binding protein, factor H-like binding proteinand vitronectin to prevent the activation of membrane attack complex on its surface. Leptospira also secretes proteases to degrade complement proteins such as C3. Leptospira can bind to thrombin that decreases the fibrin formation. Reduced fibrin formation increases the risk of bleeding. L. interrogans serovar Pomona has the SphA gene, which codes for Sphingomyelinase C that break down red blood cells. L. interrogans serovar Lai has the SphH gene, which codes for pore-forming protein and the sph2 gene where both of them damage the membranes of red blood cells.

7.8.6. Lab diagnosis

Diagnosis is done using blood, urine and CSF. Dark field microscopy and immunofluorescence microscopy are used to detect leptospira directly from sample. It is cultivated using EMJH medium using Bijou bottles. Serological diagnosis is done using microagglutination, Indirect Haemagglutination, CFT and ELISA.

7.8.7. Treatment

Leptospirosis is treated with antibiotics, such as doxycycline or penicillin, which should be given early in the course of the disease. Intravenous antibiotics may be required for persons with more severe symptoms. Persons with symptoms suggestive of leptospirosis should contact a health care provider.

7.8.8. Prevention

The risk of acquiring leptospirosis can be greatly reduced by not swimming or wading in water that might be contaminated with animal urine, or eliminating contact with potentially infected animals.

Check Your Progress

7.18. What is Weils disease

7.19. Is dark field microscopy is useful in diagnosis of leptospirosis

7.20. Mention serogroups of Leptospira

7.9. LET US SUM UP

Disease caused by Vibrio are called **Vibriosis**. *Vibrio cholerae* is a causative agent of cholera. It is a Gram-Negative Comma shaped bacilli with single polar flagellum. Cholera is a serious epidemic disease that has killed millions of people and continues to be a major health problem worldwide. Cholera is acquired by drinking water that has been contaminated with human feces or by eating food that has been washed in contaminated water. After attachment Cholera bacilli colonize on mucosal surface and produce an Enterotoxin, Cholera toxin that is responsible for Cholera symptoms. Cholera toxin **activates the adenylate cyclase enzyme in cells of the intestinal mucosa** leading to increased levels of intracellular cAMPand the secretion of H_20, Na^+, K^+,$Cl^-$and HCO_3^- into the lumen of the small intestine. The clinical description of Cholera begins after an incubation period of 6-48 hours with sudden onset of massive diarrhoea. The watery diarrhoea is also

called "**rice-water stool**" . On Tri Sodium Citrate Bilesalt Sucrose agar (TCBS) Vibrio cholera produce Circular yellow color colonies. Treatment of cholera involves the rapid intravenous replacement of the lost fluid and ions.

Salmonellosis is collective term used for invasive infections caused by a small group of bacteria called Salmonella. In Wilson Blair Bismuth Sulfite Medium, the colonies are jet black with metallic sheen colonies. Salmonellosis is transmitted by fecal oral route through contaminated food and water. In the small intestine, the bacilli attack themselves on the surface of epithelial cells of the villi and pass through them to the submucosal coat, where they are phagocytosed by Neutrophils and Macrophages. The virulent bacilli resist intracellular killing and multiply within these cells. These cells enter the Mesenteric lymphnodes, where after a period of multiplication, the bacilli invade the blood stream and primary bacteremia follows. Thus the internal organs are infected during primary bacteremia in first 7-10 days. ***Complications are*** Bleeding from intestine, Perforation of the intestine, Enteric encephalopathy, Kidney failure, Meningitis, Osteomyeletis, Loss of weight, Mental confusion. Sanitary measures and vaccines are the two main ways to prevent Enteric Fever.

Tuberculosis is one of the lower respiratory tract infection. It is a progressive granulomatous disease of the lungs. It is caused by very closely related species of Mycobacterium that are *Mycobacterium tuberculosis*. Mycobacterium has large quantities of lipids. It includes Mycolic acids (long chain fatty acid C78-C90) waxes and phosphatides. Muramyl dipeptide complexed with mycolic acid can cause granuloma formation. Phosphatide induce Tubercle formation. Lipid can cause accumulation of macrophages and neutrophiles.

Clinical signs and symptoms develop in only a small proportion (5-10 percent) of infected healthy people. Prominent symptoms are Chronic productive cough, Low-grade fever, Night sweats, Easy fatigability and Weight loss. Tuberculosis may present with or also exhibit extrapulmonary A portion of the infectious inoculum resists intracellular destruction and persists, eventually multiplying and killing the macrophage. Infection in an asymptomatic individual can be diagnosed with the help of the intradermal PPD skin test. The Mantoux test requires the intradermal injection of a measured volume (0.1 ml) containing a specified quantity (5 tuberculin units) of PPD. LJ medium is used for the cultivation of Mycobacterium tuberculosis. First-line anti-mycobacterial drugs are Isoniazid, Rifampin, Pyrazinamideand Ethambutol. BCG Vaccine is given for prevention.

Leprosy is a chronic granulomatous disease of man involving primarily the skin, peripheral nerves and nasal mucosa but capable of affecting any tissue or organs. As per WHO it is defined as a hypopigmented or reddish skin lesion with defnite loss of sensation. It is one of the Nervous system infections. *M. leprae* causes leprosy. It was the first bacilli isolated from human. It grows well on footpads of ninebanded armadillo at 30°C.

Organism enters to the human body through respiratory route or through skin. It mainly attacks nerve cells and grows very slowly in mononuclear macrophages especially the histiocytes of skin and

Schwann cells of the nerves. Rate of infection depends on the status of human immune system. Attack of immune cells against affected nerve cells produces nerve damage leading to deformity. Lepromin test is used for diagnosis. Dapsone (4,4 diamino diphenyl sulphone) is an effective monotherapy for all types of leprosy till 1982.

Pneumonia is also caused by ***Mycoplasma pneumonia*.** It is a cell wall less organism. It is resembling Pleuro Pneumonia causative agents and are called Pleuro Pneumonia Like Organisms (PPLO). Symptoms are remittent fever, coughand headache persist for several weeks. All Mycoplasmas are parasites of humans, animals, plants, or arthropods. Most Mycoplasmas that infect humans and other animals are surface parasites, adhering to the epithelial linings of the respiratory or urogenital tracts. Initial interaction between host cell and parasite releases toxic metabolites, which will cause tissue damage. Membrane fusion releases hydrolytic enzymes into the host cell leads to host cell damage. The H_2O_2 and O_2- excreted by the Mycoplasma penetrate into the host cell and cause oxidative damage. Culture is essential for definitive diagnosis. A routine mycoplasma medium consists of heart infusion, peptone, yeast extract, salts, glucose or arginineand horse serum (5 to 20%). The Mycoplasmas are sensitive to Tetracyclines, Macrolidesand the newer Quinolones. Tetracycline or Erythromycin is recommended for treatment of *M pneumoniae* pneumonia.

Leptospirosis is a relatively rare bacterial infection that affects people and animals. Several species of the Leptospira genus of bacteria cause leptospirosis. Leptospirosis is a bacterial infection. High fever, Headache, Chills, Muscle aches, Vomiting, Jaundice (yellow skin and eyes), Red eyes, Abdominal pain, Diarrhea, Rash. Leptospira enters the body through breaches in the skin or through the mucous membrane, then into the bloodstream. The bacteria later attach to the endothelial cells of the blood vessels and extracellular matrix . In the bloodstream, Leptospira can activate host plasminogen to become plasmin that breaks down extracellular matrix (ECM), degrades fibrin clots and complemental proteins (C3b and C5) to avoid opsonisation. Leptospira also secretes proteases to degrade complement proteins such as C3. Leptospira can bind to thrombin that decreases the fibrin formation. Reduced fibrin formation increases the risk of bleeding. Leptospirosis is treated with antibiotics, such as doxycycline or penicillin.

7.10. UNIT END EXERCISES

Two mark questions

- Rice water stool
- Cholera toxin
- cAMP
- TCBS
- Globi
- Hansons disease
- Define leprosy
- Nine banded armadillo
- What is tuberculoid leprosy
- Define Paucibacillary

Multibacillay
Lepromin test
PPD
Dapsone
PPLO
Crabgrass
Cellwall less bacteria
Mention causative agents of Salmonellosis

Five Mark Questions

Explain mode of action of cholera toxin
Explain method of cultivating Vibrio
Give a brief note on general characters of Vibrio.
Explain the nature of Salmonella.
Give a brief note on diagnosis of Salmonellosis.
Describe pathogenesis of Salmonellosis
Explain factors responsible for Mycobacterial pathogenisis.
Explain the symptoms of tuberculosis.
Write a note on diagnosis of tuberculosis
Describe lepromatous leprosy
Explain general features of Mycoplasma pneumonia.
Give a short note on pathogenesis and lab diagnosis of Mycoplasmal infection
What is leptospirosis? Explain
Writ about general charaters of Leptospira

Ten Mark Questions

Write a detailed note on Cholera
Explain important features of tuberculosis.
Is salmonella causes enteric infection? Explain.
Give a detailed note on Leprosy
Describe Leptospirosis.

7.11. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

7.1. Cholera
7.2. Cholera diarrhoeal stool like rice washed water so it is called as.
7.3. Venkatraman Ramakrisna medium and Alkaline peptone water.
7.4. Gram negative motile rod.
7.5. Peritrichous flagella
7.6. Blood, Urine and Stool
7.7. *Mycobacterium tuberculosis* is also called mammalion tubercle.
7.8. Virulent factor of *Mycobacterium tuberculosis*, which is responsible for chain like microscopic arrangement.
7.9. Miliary lesions, which are small granulomas, resemble millet seeds spread throughout the lung fields known as **Miliary tuberculosis**.
7.10. Usually host will begin to control the infection at some point, when primary lesion heals, it becomes fibrous and calcifies. When this happens the lesions referred to as Ghon complex
7.11. *Mycobacterium leprae is called lepra bacillus.*
7.12. Leprosy is also called Hansons disease

7.13. Nine banded armadillo is used for the cultivation of *Mycobacterium leprae*
7.14. Dapsone is a drug used for the treatment of leprosy
7.15. PPLO means Pleuro Pneumonia Like Organisms
7.16. No
7.17. Yes.
7.18. Leptospira causes hepatorenal damage thatis called Weils disease
7.19. Yes, as this is a delicate organism and spiral shaped it easily detected in dark field microscopy.
7.20. *L. icterohaemorrhagiae, L. conicola and L. hebdomadis*

4.9. SUGGESTED READINGS

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BLOCK-3
VIRAL AND FUNGAL DISEASES

UNIT VIII VIRAL INFECTIONS

NOTES

Summary

8.1 INTRODUCTION

Viruses are submicroscopic agents. They are obligate intracellular parasites. Viruses are defined as small entities whose genomes are either DNA or RNA, reproduces with in host cells. Viruses are the unique group of infectious agents. Complete viral particle are called Virion.

Unique characters of the viruses are simple acellular organization, absence of both nucleic acid, inability to reproduce independenly and absence of cell division. As viruses are obligate intracellular parasites, they are multiplied within the cell and disturbs cell and leads to diseases.

8.2 OBJECTIVES

Learnerns will be able to understand

Disease causing viruses.
Burden caused by viral disease.
Importance of lab diagnosis
How viruses cause a disease
Treatment of viral disease.

8.3. INFLUENZA

8.3.1. Introduction

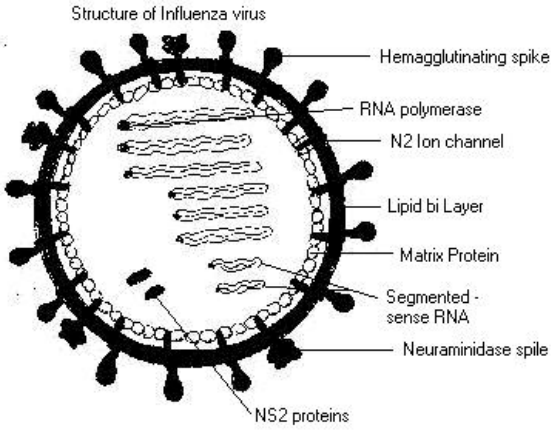
It is a viral infection of the respiratory tract cause fever, headache, muscle ache and weakness. Orthomyxo virus family member Influenza virus causes this disease. The name myxo virus was proposed originally for a group of enveloped RNA viruses characterized by their ability to absorb into mucoprotien receptors on erythrocytes, causing hemagglutination.

8.3.2. History

Influenza is an acute infectious disease of the respiratory tract, which occurs in sporadic, epidemic and pandemic form. Italians gave the name influenza during the year 1358. In 1933, Smith isolated the causative agent (Influenza A). Burner (1935) developed chick embryo technique for propagation of virus. Francis and Magill (1940) isolated a serotype of

the influenza and named influenza B. Taylor (1949) isolated the third serotype of influenza virus, type C.

8.3.3. Causative Agent



Influenza Virus causes Influenza. It belongs to Orthomyxo virus family.

It consist of three species, they are A, B and C. Type A usually responsible for the large outbreaks and is a constantly changing virus. New strains of type A virus develop regularly and results in a new epidemic every few years.

Type B and C is fairly stable virus.

Type B causes smaller out breaks. Type C usually cause mild illness like common cold. Influenza virus are spherical and 80-120nm in diameter. Antisense RNA genome occurs in 8 separate segments containing 10 genes. The segments are complexed with nucleoprotein to form nucleocapsid with helical symmetry. Nucleocapsid is enclosed in an envelope consisting of a lipid bilayer and two surface glycoproteins, a hemagglutinin and neuraminidase. Readily inactivated by non-polar solvents and by surface-active agents. Influenza C virus having 7 segments of RNA and only one surface protein. The virus inactivated by heating at 50°C for 30 minutes. It remains viable at 0-4° for about a week. Infectivity lost rapidly at 20°C. Preserved at –70° or by freeze - drying. Membrane protein is known as matrix protein or M protein. M2 protein projects through the envelope to form ion channel. It is responsible for gene transfer. H gene is responsible for hemagglutinin spike and N gene responsible for Neuraminidase. Spikes measures about 10mm in length and molecular weight of 225000 Dalton.

Gene responsible for viral protein

10 genes from 8 segments of antisense RNA responsible for synthesis.

8.3.4. Symptoms

Fever
Cough with or without mucous
Nasal discharge
Headache
Muscle ache and stiffness
Shortness of breath
Chillness
Sweating
Fatigue
Abnormal taste
Malaise
Stuffy and congested nose
Sore throat
Clammy skin
Nosebleed
Vomiting
Joint Stiffness
Elbow pain
Loss of appetite

8.3.5. Pathogenesis

Influenza virus is transmitted from person to person primarily in droplets released by sneezing and coughing. It is highly contagious disease. The incubation period for influenza is 1-4 days. Alveoli is the primary target for virus. Infected cells will sloughs, allowing extravagation of fluid and secondary submucosal inflammation. During initial stage it liquefies mucous and attaches other cells. The infection in mucosal cell results in cellular destruction and desquamation of the superficial mucosa. The resulting odema and mononuclear cell infiltration of the involved areas are accompanied by symptoms like cough, sore throat and nasal discharge. Most of the symptoms are because of interferons. Current evidence indicates that the extent of virus induced cellular destruction is the prime factor determining the occurrence. In an uncomplicated case, virus can be recovered from respiratory secretions for 3-8 days. The disease may extensively involve the alveoli, resulting in interstitial pneumonia, sometimes with marked accumulation of lung hemorrhage and odema.

8.3.6. Multiplication

Virus replication takes about 6 hours and kills the host cell. The virus enters permissive cells via the hemaggultinin subunit, which binds to cell membrane glycolipids or glycoprotein containing sialic acid or N-acetylneuraminic acid, the receptor for virus adsorption. The virus is then engulfed by pinocytosis into endosomes. The acid environment of the endosome, uncoating the nucleocapsid and releasing it into the cytoplasm. A transmembrane protein derived from the matrix gene forms an ion channel for protons to enter the virion and destabilize protein binding allowing the nucleocapsid to be transported to the nucleus, where the genome is transcibed by viral enzymes to viral mRNA. Unlike replication of other RNA viruses, orthomyxo virus replication depends on the presence of active host cell DNA. The synthesized viral mRNA are transported to the cytoplasm, where it translated by host ribosome.

mRNA's specifying viral membrane proteins (HA,NA,M) are translated by ribosome bound to endoplasmic reticulum and they undergoes glycosylation. The nucleocapsid is assembled in the nucleus. After the attachment of M1 protein to newly synthesized RNA, viral RNA synthesis is stopped and nucleocapsids are transported out. HA and NA proteins are transported to the cell surface and are incorporated into the plasma membrane. Virion nucleocapsids along with NS2 associate with regions of plasma membrane containing HA and NA proteins. After acquiring envelop and undergo maturation as they bud through the host cell membrane.

During budding, the viral envelope hemagglutinin is subjected to proteolytic cleavage by host enzymes. It had 3 basic amino acid, which is specifically attack by host enzymes. These are called Taubenberger amino acid. This process is necessary for the released particles to be infectious.

8.3.7. Gene Reassortment

Because the influenza virus genome is segmented, genetic reassorment can occur when a host cells is infected simultaneously with viruses of two different parent stains. If a cell is infected with two strains of type A virus, for example, some of the progeny virions will contain mixture of genome segments from the two strains. This process may leads to Influenza pandemics. This process is also called antigenic shift. It is the major antigenic change. Smaller antigenic change is called antigenic shift.

8.3.8. Epidemiology

Influenza viruses are classified as types A,B,C on the basis of antigenicity of their nucleoproteins and matrix protein. Rainy season is a peak time for influenza. Influenza epidemic is of two types. Both type A and type B viruses cause yearly epidemics. Type A caused influenza pandemics. Two different mechanisms of antigenic change are responsible for producing the strains that cause these two types of epidemic. Some of the Influenza strains were transmitted from animal to humans. Mostly it is transmitted through person-to-person contact and also droplet spread.

8.3.9. Laboratory Diagnosis

The most commonly employed method for laboratory diagnosis is recovery of the virus from specimens containing respiratory secretions, such as nasal wash and throat swab or sputum.

Methods Include Isolation of virus, Cold agglutination, Influenza complement fixation, Immunoflourescent technique

Isolation of Virus

Respiratory secretions are treated with antibiotics and inoculated into amniotic cavity of 10-11 day old egg or monkey kidney cells. Incubate at 35°C for 3 days. Then eggs were chilled and harvest amniotic fluid. The presence of viral antigens are demonstrated by using hemadsorption test at 4°C. Type B Agglutinate both Guinea and Fowl cells. Type C agglutinate only fowl cells.

ELISA also useful for demonstration of antigens. Serological examination is by specific antigen and antibody reaction.

8.3.10. Complications

Reye's Syndrome

It is an acute encephalopathy of children of 2-16 years. Fatty degeneration of liver is associated with this syndrome. Mortality rate is 10-40%. Otitis media, sinusitis, asthma, bacterial pneumonia and Aspergillosis are the other symptoms

8.3.11. Prevention

Inactivated influenza virus vaccines have been used for old age people. The virus for the vaccine are grown in chick embryo, inactivated by formalin, purified to some extent and adjusted to a dosage known to elicit an antibody response in most individuals.

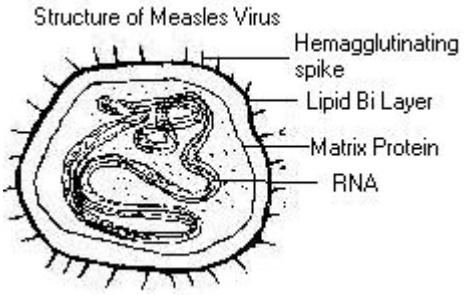
8.3.12. Treatment

The synthetic drugs Amantadine and Rimantadine hydrochloride effectively used to prevent infection and illness caused by type A and but not by type B viruses. The drugs interfere with virus uncoating and transport by blocking the trans membrane M2 ion channel. Drugs prevent about 50-67% of infection. Drug resistance also occurs.

Check Your Progress
8.1. What is orthomyxo virus
8.2. Who isolated Influenza virus A
8.3. What is antigenic shift
8.4. What is antigenic drift
8.5. Explain Ryes syndrome

8.4. MEASLES

8.4.1. Introduction



Measles is a highly contagious skin disease that is epidemic throughout the world. Measles virus, a member of the genus Morbili virus and the family Paramyxoviridae. Thomas Sydenham in 1690 gave the first clear and accurate description about Measles. In 1846 an outbreak of Measles occurred in remote areas of Islands. Gold Berger and Anderson established the viral etiology of Measles in 1911 by transmitting the disease to Monkey through the inoculation of filtrates of blood and nasopharyngeal secretions.

8.4.2. Causative agent

Measles is caused by a pleomorphic, medium sized (120-200 nm in dia) virus. It comes under Paramyxoviridae family. Its genome is RNA. It is an enveloped virus. It has two biologically active projections one 'H' is responsible for viral attachment to host cells and causes hemagglutinations. The outer 'M' is responsible for fusion of the viral outer membrane with the host cell. The M antigen also responsible for producing multi nucleated giant cells. It has tightly coiled nucleic acid surrounded by the lipo protein envelope. The virus grows well on human or monkey kidney and human amnion culture, which are the preferred cells for primary isolation. The other name for multinucleated giant cells is ***war thin-finkeldey*** cells. The virus is heat labile and readily inactivated by heat. UV rays, ether and formaldehyde.

8.4.3. Symptoms

Incubation period is about 10-12 days. It begins with fever, runny nose, cough and swollen weepy eyes. Within a few days, a fine red rash appears on the fore head and spread outward over the rest of the body. Unless complications occur, symptoms disappear within one week.

Unfortunately many cases are complicated by secondary infections caused by bacterial pathogens, mainly *Staphylococcus aureus*, *Streptococcus pneumoniae, Streptococcus pyogenes* and *Haemophilus influenzae.*

Very rarely, Measles reactivation is observed after two to ten years and forms a disease called **Subacute Sclerosing Pan Encephalitis (SSPE)**, which is marked by slow progressive degeneration of the brain, resulting in death within two years.

Measles occurs during pregnancy results in an increased risk of miscarriage, premature labor and low birth weight. Protracted diarrhoea is often seen as a complication in children in poor nations.

8.4.4. Pathogenesis

The respiratory route and conjunctiva acquire Rubeola virus. It primarily replicates in the upper respiratory epithelium then spreads to lymphoid tissues and following further replication eventually spreads throughout the body. Mucous membrane involvement is responsible for an important diagnostic sign **Koplicks spot** (small bluish white ulceration on the buccal mucosa). Damage to the respiratory mucous membrane partly explains the markedly increased susceptibility of Measles patients to secondary bacterial infections, especially infection of the middle ear and lung.

The skin rash of measles results from the cytopathic effect of Rubeola virus replication in skin vascular endothelial cells and cellular immune response against the viral antigen in the skin. It is not known why the rash characteristically outward, often clearing on the face before it reaches the lower parts.

The measles virus temporally suppress the cellular immunity, which can cause reactivation of Herpes Simplex Virus

8.4.5. Laboratory Diagnosis

Primary diagnosis is with the help of Kopliks spot formation.

Sample - Throat/Nasopharyngeal swab, Urine , Whole blood

Turn around time: 14 days

Cytologic Diagnosis- Specimens should be fixed with formalin and stained with Hematoxylin and Eosin. Characteristic giant cells containing eosinophilic intranuclear and intracytoplasmic inclusions are observed for the first 2 or 3 days of the specimen.

Antigen Detection- It is with Immunofluorescence technique, immuno enzyme staining increase the sensitivity of the test.

Virus Isolation - Isolated by cell culture technique from respiratory secretions and other samples. Primary cultures of human embryonic kidney cell and monkey kidney cells are more sensitive for viral isolation.

Nucleic Acid Detection - This technique was done in immunocompromised patients who maynot be capable of antibody

response. Viral nucleic acid is detected by using reverse transcriptase, PCR insitu hybridization or reverse transcriptase, PCR and Amplification of RNA extracted from specimens.

8.4.6. Epidemiology

Humans are the only natural host for Rubeolla virus. It is eradicated from US but occasionally epidemics were observed.

NOTES

8.4.7. Control

Children too young to be vaccinated. MMR vaccine is used. Preschool children also vaccinated.

Check Your Progress

8.6. What is Koplic spot

8.7. What is Warthin Finkeldey cells.

8.8. SSPE

8.9. Is there any Vaccine available for measles

8.5. MUMPS

8.5.1. Introduction

It results from an acute viral infection. Target of Mumps is parotid gland, is located just below and in front of the ear. Mumps means mumble. Mumps begins with painful swelling of one or both parotid gland.

8.5.2. Causative Agent

Mumps virus causes Mumps. It is an enveloped virus included under the family Paramyxoviridae. It is a ssRNA containing negative sense virus. It is a helical shaped virus. Viral etiology was demonstrated by Johnson and Goodpastuer in 1934. Hebel cultivated it in embroyonated eggs. In 1955, Henle and Deinhardt grew it in tissue culture. Virus posses hemagglutinin, neuraminidase and fusion protein. It is a heat labile and chemically sensitive virus.

8.5.3. Symptoms

Incubation period – 16 to 18 days. Parotid swelling with pain is the first sign. Fever, extreme pain during swallowing.

8.5.4. Pathogenesis

Virus is transmitted in saliva and respiratory secretionsand its portal of entry is respiratory tract. This virus multiplies in the respiratory tract and local lymphnode in the neck. Virus spreads throughout the body by the blood stream and produces symptoms only after infecting other tissues such as the parotid glands, meninges. In the salivary gland, the virus multiplies in the epithelium of ducts that convey saliva to the mouth. This destroys the epithelium. The body inflammatory response to the infection is responsible for the severe swelling and pain. In adults it infect tubule and cause death of testicular tissue. The immune system of the host eliminates the infection.

8.5.5. Epidemiology

Humans are the only natural host of Mumps and natural infection confers life long immunity.

8.5.6. Lab Diagnosis

Generally serological diagnosis is not necessary. This virus can be identified with hemagglutination inhibition test. Embryonated eggs and cell culture techniques are used for culturing.

8.5.7. Control

An effective vaccine is a variable and is often administered as part of the trivalent Measles, Mumps and Rubella (MMR) vaccine. It provides protection for at least 10 years.

Check Your Progress

8.10. What is mumps

8.11. Name the protein present in Mumps virus.

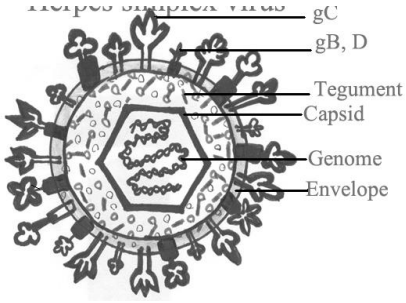
8.6. CHICKEN POX

8.6.1. Introduction

Chicken pox is a common childhood infection. It is a mild, highly contagious disease, chiefly occurs in children, characterized by a generalized vesicular eruption of the skin and mucous membrane. Chicken pox is caused by Varicella virus. It belongs to herpes viridae family.

8.6.2. Causative agent

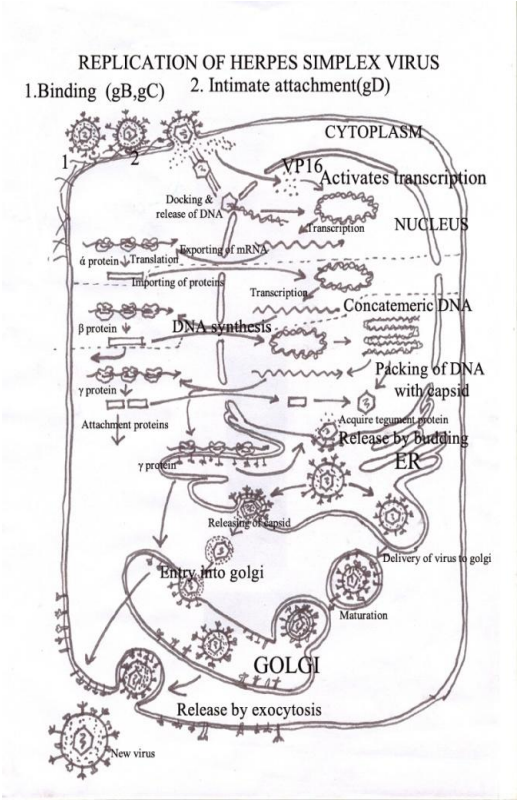
Varicella viruses are large viruses.Virion is spherical shaped enveloped virus. Capsid is Icosahedral in shape. Genome is linear double standard DNA, 124-235kbp. More than 35 proteins are available in virion. Virion Replicates is in the nucleus. Genome is large enough to code for at least 100 proteins.



8.6.3. Replication

- Virion binds to the extracellular protein through gB and gC receptor.
- Another viral protein gD interacts with a second cellular receptor.
- This interaction mediates fusion of virus with host plasma membranes.
- The virus is uncoated, liberating tegument proteins and nucleocapsid into the cytoplasm.

- Viral nucleocapsid docks at the nuclear pore and release viral DNA into the nucleus, where the DNA circularizes.
- VP16 enhance transcription of viral genome and stimulate transcription of immediate early genes by host cell RNA polymerase II.
- Immediate early mRNAs are spliced and transported to the cytoplasm, where they are translated.
- Immediate early proteins (α proteins) are imported into the nucleus, where they activate the transcription of early genes.



- β protein genes are transported to the cytoplasm after transcription and are translated. β proteins are imported to the nucleus where they induce DNA replication and synthesis substrate for DNA synthesis.
- DNA replication produces long concatameric DNA molecules, the templates for late gene expression.
- Late mRNAs are transported to the cytoplasm and synthesis of gamma protein. These proteins are structural proteins and are needed for viral assembly.
- Some late proteins are inserted to ER and are transported to Golgi apparatus for glycosylation.
- Mature glycoproteins are transported to plasma membrane of the infected cell.
- Some gamma proteins are transported to the nucleus for assembly of nucleocapsid and DNA packaging.
- Newly replicated viral DNA is packaged into preformed capsids.
- These capsids, together with some tegument proteins bud from the inner nuclear membrane into the lumen of ER and acquire envelope.
- Enveloped virus then transported to the PM for release by exocytosis.
- Latent infection occurs primarily in neurons found in sensory and autonomic ganglia. During this infection Latency Associated Transcript (LTT) promoter is synthesized and are involved in protein synthesis.

8.6.4. Pathogenesis

The route of infection is the mucosa of the URT or the conjunctiva. The virus circulates in the blood and undergoes multiple cycles of replication and eventually localizes in the skin. Lesions of varicella infection are associated with cutaneous and mucosal endothelial cells. Swelling of epithelial cells, ballooning degeneration and the accumulation of tissue fluids results in vesicle formation. Eosinophilic inclusion bodies are found in the nuclei of infected cells. Multinucleated giant cells are common.

Zoster lesions are histopathologically similar to varicella. There is also an acute inflammation of the sensory nerves and ganglia. It is not clear what triggers reactivation of latent Varicella-Zoster virus infection in ganglia. It is believed that warning immunity allows viral replication to occur in a ganglion, causing intense inflammation and pain.

8.6.5. Symptoms

Incubation period is of 10-23 days. Malaise and fever are earliest symptoms. Followed by rash characteristically begins on the scalp and trunk and spreads. Macules evolve in 2-3mm vesicles, evolve in successive crops. Lesions on mucous membranes are easily transmitted and may appear as ulcers. Lesions appear on the Mouth, Rectum and Vagina. Other symptoms include Headache, Sore throat, Loss of appetite and Irritability.

Zoster infection usually starts with severe pain in the area of skin or mucosa supplied by one or more groups of sensory nerves and ganglia. The most common complication of Zoster is **Post Herpatic Neuralgia (PHN).** Pain may be characterized by burning, itching or tingling sensations.

8.6.6. Lab Diagnosis

Demonstration of multinucleated giant cells and type A intranuclear inclusion bodies. CF, Neutralization test are used a serological test

8.6.7. Treatment

Vidarabine and Acyclovir are useful for treatment. Zoster Immuno Globulin (ZIG) also useful.

Check Your Progress

8.10. What is mumps

8.11. Name the protein present in Mumps virus.

8.12. What is Post Hepatic Neuralgia

8.13. Mention Eosinophilic inclusion of chicken pox

8.7. HEPATITIS A, B, C, D & E

8.7.1. Introduction

Hepatitis is the term used for any condition where there is inflammation or necrosis of liver cells. Necrosis is the term used for death of some or all cells in an organ or a tissue. Viral hepatitis has emerged as a major public health problem throughout the world affecting

several hundreds or millions of people. Hepatitis and Jaundice are not same. Jaundice is the term used for yellow discoloration of Sclera, the white of the eyes. Jaundice is due to various reasons, one of the reason is Hepatitis.

Some viral groups that are called Viral Hepatitis cause damage of liver. Hetrogenous group of viruses that are called Hepatotrophic viruses causes it. These includes,

Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus(HCV), Hepatitis D virus(HDV), Hepatitis E virus (HEV).

The only common feature of these Hepatitis viruses is their primary Hepatotrophism.

On the basis of epidemiology and clinical criteria, Hepatitis was classified into two. It is also differentiated on the basis of serology and molecular markers. One type occurred sporadically or as epidemics, affecting mainly children and young adults and transmitted by fecal oral route. This was called *Infective or Infectious Hepatitis*, later termed type A Hepatitis.

A second type of viral Hepatitis transmitted by serum inoculation or blood transfusion. This was called in various names such as *Homologous serum Jaundice, Serum Hepatitis and transfusion Hepatitis*. It was latter called type B Hepatitis. All infectious Hepatitis caused by type A viruses. All serum hepatitis caused by type B viruses. About 98% of Hepatitis is caused by Hepatitis A,B,C,D and E viruses. Exact mechanism for liver cell damage is not well understood. But it may through Complex process of body's defence mechanism. By direct attacking and damaging of liver cell.

8.7.2. HEPATITIS A VIRUS

8.7.2.1. Introduction

Hepatitis type A is a subacute disease of global distribution, occurring mainly in children and young adults. The term infectious hepatitis was coined in 1912 to describe the epidemic form of the disease.

8.7.2.2. Characters

HAV was first demonstrated by Feinstone and coworkers during the year 1973 from feces through Immuno electron microscopy. It is a 27nm nonenveloped, symmetrical (+) RNA virus. Most of its character mimics Picornovirus family. It was designated as enteroviruses type72. It is non cytopathic when grown in cell culture. It cause necrosis of paranchymel cells and histiocytic perifortal inflamation invivo. Icosahedral symmetry. It was classified into the Enterovirus during the year 1983. HAV genome comprises about 7500 nucleotides. The virus is relatively resistant to inactivation. Virus withstand 60°C for 1 hour and With stand 100°C for 1 minute. Inactivated by formaldehyde 1:4000 at 37°C for 72 hours. Chlorine 1ppm in 30 seconds. It is not affected by non ionic detergents. It stands prolonged at 4°C or cooler temperatures.

8.7.2.3. Pathogenesis

Clinical expression of HAV varies considerably. HAV enters body via ingestion of contaminated food, water and fecal oral route.

Enter through fecal oral route, Food and water
↓
Replicates in Oropharynx and epithelial lining by intestine.
↓
Withstand stomach acidity because of non enveloped virus
↓
It crosses intestinal barrier and reaches liver and binds the receptors of liver parenchyma cells.
↓
Produce symptoms
↓
Virus released and enters the Intestine through bile.
↓
Release outer environment through feces 10 days before starting of symptoms

Intestine→ Liver → Bile → Intestine→ Feces → Oral.

The virus spread from intestine to liver through blood stream. Incubation period for HAV is 3-5 weeks within a mean of 28 days.

8.7.2.4. Symptoms

Fever, Malaise, Anorexia, Nausea, Vomiting, Liver tenderness. Mortality is very low ranging from 0.1-1%. General symptoms subside with the onset of Jaundice.

8.7.2.5. Lab Diagnosis

Isolation of virus in tissue culture requires prolonged adaptation and it is, therefore, not suitable for diagnosis.

Serological techniques are available, that includes, Immuno electron microscopy, Complement fixation, Immune adherence hemagglutination, Radio immuno assay and Enzyme immuno assay.

8.7.2.6. Control and Treatment

Improve sanitation, Prevent fecal contamination, Prevent direct contact with infected individuals. Available vaccines are, Inactivated vaccines (formaldehyde)- Intra muscular; Attenuated vaccine – Oral; Post exposure treatment include Human immunoglobulin and anti HAV.

8.7.2.7. Prevention

Drink water only from safe drinking water source, Use boiled water while brushing your teeth when you are travelling in an area where the risk of getting HAV virus, Avoid eating fruits, salads or uncooked vegetables that have not been washed by you in treated or boiled water. Avoid eating food or drinking beverages from street venders, especially if they are not covered. Take HAV vaccine if you are high risk getting the infection.

8.7.3. Hepatitis B Virus

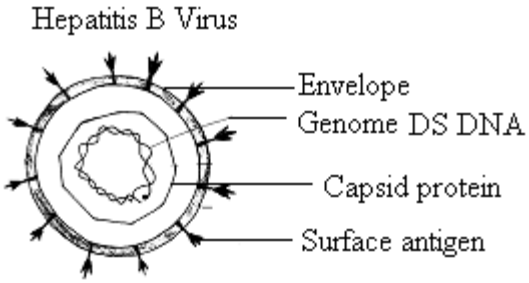
8.7.3.1. Introduction

It is the most important type among Hepatitis causing viruses. HBV causing Hepatitis is called Serum Hepatitis. The disease occurs throughout the world. It is an important cause of acute and chronic infection of liver. HBV infection causes more than million-deaths per year worldwide.

In 1965, Blumberg reported protein antigen in the serum of Australian patient. This antigen is called Australian antigen.

8.7.3.2. Causative agent

By 1968, the Australian antigen was shown to be associated with Serum Hepatitis. This was then considered as Hepatitis surface antigens(HbsAg). HBV is a 42nm spherical virus that posses several antigens. There are three envelope polypeptides that come under the designation HbsAg, HbcAg & HbeAg. HBV belongs to Hepadna viridae.



The nucleocapsid of the virion consist of the viral genome surrounded by the core antigen.(27nm). The genome, which is approximately 3.2 KB in length, has an unusual structure and is composed by two linear strands of DNA held in circular configuration. Negative strand is complete but positive strand is incomplete. 3' end of genome is associated with a DNA polymerase molecule. 22nm Dane particle and, tubular structure combined to form HbsAg.

In genome there are 4 major Open reading frame (ORF)

ORF-S ⇒ Consists of pre S1and pre S2. – Codes for structural proteins of surface and core.

ORF-P ⇒ Encodes polymerase that contains DNA polymerase & RNase H.

ORF-X ⇒ Encoded transcriptional activator.

ORF-C⇒ Pre C protein.

The virus is stable at 37°C for 60 minutes. Destroyed its antigen by 0.5% sodium hypochlorite for 3 minutes. HbsAg stable at pH2.4 for 6 hours. 2% glutaraldehyde destroy antigenicity within 3 minutes. HbsAg is resistant to UV. Virus did not grown in tissue culture medium.

Complications

- Pain in liver,
- Liver cancer,
- Hepato cellular carcinoma,
- Arthralagia,
- Polyarteries,
- Glomerulonephritis.

8.7.3.3. Symptoms

Incubation period varies widely from 40days-6months, but is often about 2-3 months.

Discomfort in feeling, Tiredness, Fever,

Chills, Loss of appetite, Nausea, Vomiting, Headache, Pain in abdomen, Diarrhoea, Jaundice, Itching sensation on skin, Urine become dark, Light colored stool, Inflammation in joints.

Mortality rate is about 0.5-2%

Most of the peoples develop Carrier State.

8.7.3.4. Pathogenesis

Pathogenesis involves 3 steps

Entry

Multiplication and spread

Liver cell damage.

Entry

Following means performs transmission of virus

Blood transfusion.

Sexual transmission.

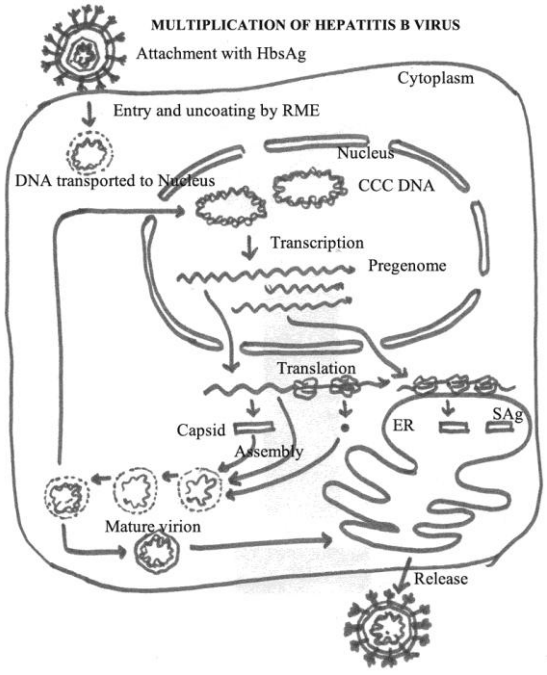
Neonates get infected from mothers.

Through contaminated syringes and needles.

In rare case, it is by arthropod's.

2. Multiplication and spread

The target of HBV is the hepatocyte. HBV proteins and genomes were identified in extrahepatic sites also (Bone marrow, spleen, lymphnodes and circulating lymphocytes). But it didn't produce any damage in these locations.



Virus attaches to the hepatocyte with the help of surface antigen (HbsAg).

HBV enter inside of cytoplasm through receptor mediated endocytes.

DNA of the virion is transported into the nucleus.

DNA transcription follows resulting in the formation of mRNA.

Short mRNA transcripts undergoes translation in ER.

Translation products are preS and S proteins.

P protein translation occurs in the cytoplasm and it is coupled to capsid protein formation.

P and capsid proteins were assembled and cover DNA.

Replicated DNA undergoes nick formation resulting in dsDNA with staggered strand.

Morphological changes occur on the surface of plasma membrane.

Complete virions released through exocytosis.

Replication results in injury of hepatocytes and release of progeny virions into the blood stream. Cell injury is not only caused by cytopathic effect of virus and also caused by the activation of cytotoxic immune

mechanisms. This will results in liver tissue degeneration and the release of liver associated enzymes into the blood stream.

This is followed by jaundice, the accumulation of bilirubin in the skin and other tissues with a resulting yellow appearance.

CMI may be important in terminating the infection and in some instances it will causes immune mediated liver damage. Exogenous interferon maybe effective in treating some patients with chronic hepatitis.

Chronic hepatitis leads to persistent hepatitis, with mild periportal inflammation. This will leads to Chronic Active Hepatitis (CAH) with more widespread inflammation and necrosis. CAH lead to cirrhosis and hepatocellular carcinoma.

8.7.3.5. 1Laboratory Diagnosis

Several types of blood tests are recommended for diagnosis of Hepatitis.

Liver enzyme - Blood levels of liver enzymes such as alanine aminotransferase and a aspartate aminotransferase are elevated in the early stages of viral hepatitis.

Hepatitis B antigens - ELISA test is used for the detection of HbsAg . It is also used for the detection of Hepatitis antibodies.

8.7.3.6. Epidemiology

140,000-320,000 infection/year
70,000-1,60,000 symptomatic infections.
Out of symptomatic infections about 8400-19,000 Hospitalization/year and 140-320(0.2%) deaths/year.
Of all infections, 8,000-32000 chronic/year and 5000-6000 death/year.
Intermediate endemicity observed in India.
5-10% adult act as carrier.
30% children act as carrier.
90% neonates act as carrier.
350 million carriers in worldwide, out of these, 45 millions are in India.

8.7.3.7. High risk groups for HBV

Health care workers who come in contact with contaminated blood or other body fluids. Male homosexuals. People having sexual contact with those whom have HBV. People with kidney diseases that require dialysis. People who receive organs for transplant. People undergoing treatment for leukemia. Babies born from infected mothers. Intravenous drug uses.

8.7.3.8. Prevention

Prevented by active and passive immunization. Two types of vaccine currently available. Recombinant HB vaccine-synthesized from yeast cells-safe and effective-provides 90% protection. Plasma derived vaccine. Vaccine injection was given in muscles of the upper and outer parts of the arm at birth, at the age of one month and at 6 month. A booster dose is recommended at 5 **years of age.**

8.7.3.9. Treatment

People with chronic and active inflammation of liver cells due to HBV are treated with Interferon. It blocks the infection of virus into the cells. In recent years interferon is prescribed in combination with ribavarin. Interferon is not recommended for people who have developed cirrhosis or scarring of liver tissue. During this time lamuvidine is recommended. Medicine decreases the multiplication of virus.

8.7.4. Hepatitis C Virus

HCV is the leading cause of post transfusion hepatitis. Both chronic, asymptomatic carriers and chronic hepatitis have been documented with HCV. Frequency of HCV is greater than HBV. Post transfusion chronic hepatitis may occur in upto 54% of cases. Parentally transmitted nonA, nonB hepatitis, now known as HCV, can be identified by a specific serologic test for anti-HCV antibodies. This virus is 30-60nm,spherical shaped, positive sense RNA virus. Genome has 10,000 nucleotides. HCV is considered as the major risk factor because 80% of infections lead to chronicity. 70% leads to chronic active hepatitis or cirrhosis. Infection is also associated with progression to primary liver cancer and hepatocellular carcinoma. HCV rarely seems to cause fulminant hepatitis. The genome of HCV resembles those of the Pestivirus and Flavivirus. All genomes contain a single large open reading frame, which is translated to yield poly proteins from which the viral protein were derived by post transnational cleavage and other modification. Helicase, polyproteins and proteases were involved in RNA replication.

8.7.5. Hepatitis Delta Virus

HDV occurs only those who have HBV infection. In 1977, Rizzetto & colleagues in Italy identify a new viral antigen in the liver cell nuclei of patients infected with HBV. Later it was called HDV. HDV is coated with HbsAg, which is needed for release from the host hepatocyte and for entry in the next round of infection. HDV is a spherical, 36nm particle with an outer coat composed of HBV, surface antigen surrounding the circular ssRNA with 1.7-kb base pairs. The internal protein molecular weight 68,000. Closest relative of HDV is a satellite virus of plants. Two types of infections were recognized. Co infection-HDV&HBV are transmitted together at the same time. Super infection-Delta infection occurs in a person already harboring HBV. HDV has 1679 nucleotides. RNA replicates with the help of host RNA polymerase II. About 5% HbsAg carries worldwide are infected with HDV. Diagnosis is performed with immunofluorescence and ELISA. An IgM antibody appears 2-3 weeks after infection and is soon replaced by IgG antibody.

8.7.6. Hepatitis E Virus

It is an single stranded linear RNA virus. It included under the family Calciviridae. Largest epidemic occurred in Delhi during the winter of 1955-56, affecting over 30,000 people within 6 weeks. Incubation period ranges from 2-9 weeks with an average of 6weeks. Most cases occur in the young to middle aged adults (15-40year). A unique feature is the clinical severity and high case fatality rate of 20-

40% in pregnant women, especially in the last trimester of pregnancies. HEV is a spherical non-enveloped virus, 30-32nm in diameter. It enters liver through intestine and blood. Identified by means of ELISA. There is no vaccine currently available for preventing HEV. Relapse of HEV infection is common. Personal hygiene and sanitation are the only effective way of prevention.

8.7.7. Differentiation properties of Hepatitis Virus

S. no	Characters	HAV	HBV	HCV	HDV	HEV
1	Family	Picornaviridae	Hepadnaviridae	Flaviviridae	Defective Virus	Calcivirus
2	Genus	Enterovirus	Hepadnavirus	Hepacavirus	Deltavirus	Hepevirus
3	Size	25-29nm	40-50nm	30-60nm	35nm	30-32nm
4	Capsid	SS RNA(+)	DS DNA	SS RNA(+)	SS RNA(-)	SS RNA(+)
5	Nucleic Acid	Icosahedral	Spherical	Spherical	Spherical	Icosahedral
6	Virion	Nonenveloped	Enveloped	Enveloped	Enveloped	Nonenveloped

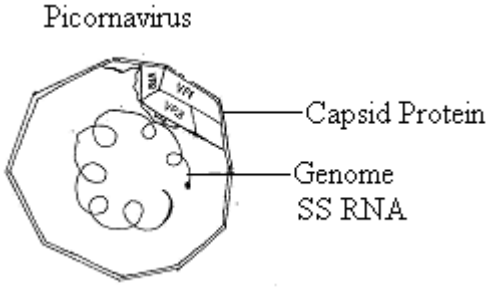
Check Your Progress

8.14. What is Australian antigen
8.15. Name the person who discovered HBV.
8.16. Name closest relative of HDV
8.17. What is Co-Infection

8.8. POLIOMYELITIS

8.8.1. Introduction

Polio is an ancient disease. Various Egyptian hieroglyphics dated approximately 2000B.C depict individuals with wasting, withered legs and arms. In 1840 the German orthopedist Jocob Von Heine described the clinical features of Poliomyelitis and identified spinal cord as the problem area. Poliovirus has tropism for epithelial cells of the alimentary tract and cells of the central nervous system. Infection is asymptomatic or causes a mild, undifferentiated febrile illness. Spinal and bulbar poliomyelitis occasionally occurs. Paralytic poliomyelitis is not always preceded by minor illness. Paralysis is usually irreversibleand there is residual paralysis for life.

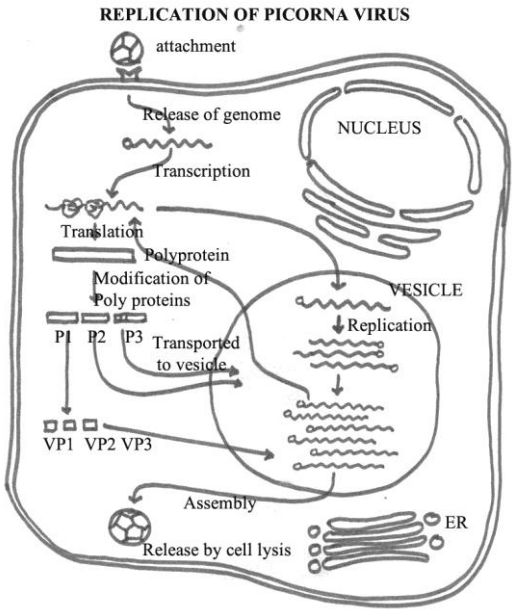


8.8.2. Causative Agent

All three Poliovirus serotypes (1 to 3) can give rise to paralytic poliomyelitis. It is a nonenveloped virus. It is included under the family ***Picrno Viridae***. The *Picornaviruses* are (naked), small (22 to 30 nm) *icosahedral* virions *resistant to lipid solvents*. The virus capsid is composed of 60 copies

each of four viral proteins VP1 to VP4, which form an icosahedral shell. The genome is a *single stranded RNA* (molecular weight, approximately 2 x 106 to 3 x 106). The RNA strand consists of approximately 7,500 nucleotides and is covalently bonded to a *noncapsid viral protein (VPg) at its 5' end* and to a polyadenylated tail at its 3' end.

Enteroviruses can survive for long periods in organic matter and are resistant to the low pH in the stomach (pH 3.0 to 5.0). Picornaviruses are inactivated by pasteurization, boiling, Formalinand chlorine.



8.8.3. Multiplication

The virus binds to a cellular receptor. The mechanism of uncoating of RNA genome are unknown. VPg protein at the 5' end is removed form RNAand the resulting RNA associates with ribosomes. Translation is initiated at an internal site of 741 nucleotides from the 5' end of the viral RNA and poly protein precursors are synthesized.

Polyproteins are cleaved and produce individual proteins (P1,P2 and P3)

P1 proteins contain viral structural proteins.

P2 and P3 are responsible for proteases and RNA synthesis proteins.

The proteins that involve in RNA synthesis are transported into the membranous vesicles.

Positive sense RNA also transported into the vesicles.

It is copied into minus sense RNA that is the template for the synthesis of positive sense RNA.

Structural proteins are formed by partial cleavage of P1 precursor proteins.

These proteins are transported to vesicles

Assembly takes place within the vesicles.

Mature virions released after cell lysis.

8.8.4. Symptoms

Paralytic poliomyelitis can occur without antecedent minor illnesses. A patient may suffer aseptic **meningitis with pains in the back and neck muscles** for several days.

8.8.5. Pathogenesis

Incubation period is about 7-14 days. Humans are the only natural host of Poliovirus. They attach to a specific receptor on these cells, which in humans is encoded by a gene on chromosome 19. Poliovirus infection is quite common in nonimmunized individuals, but only about 1 percent of these cases progress to the paralytic form of the disease. Primary replication of Poliovirus takes place in the oropharyngeal and intestinal

mucosa (the alimentary phase). From here, the virus spreads to the tonsils and Peyer's patches of the ileum and to deep cervical and mesenteric nodes, where it multiplies abundantly (the lymphatic phase). Subsequently, the virus is carried by the bloodstream to various internal organs and regional lymph nodes (the viremic phase). More concentrated damage results in flaccid paralysis of the muscles innervated by the affected motor nerves. Muscle involvement peaks a few days after the paralytic phase begins. Paralysis is usually irreversibleand residual paralysis remains for life. Paralytic disease is called spinal poliomyelitis if the weakness is limited to muscles innervated by the motor neurons in the spinal cord and bulbar poliomyelitis if the cranial nerve nuclei or medullary centers are involved.

8.8.6. Epidemiology

Poliomyelitis affects all age groups. In areas with poor hygiene and poor sanitation, most infants are infected relatively early in life and acquire active immunity while still protected by maternal antibodies. In countries, which have high poliomyelitis immunization, coverage in their childhood vaccination programs there is a shift to an older age group.

8.8.7. Lab Diagnosis

Enteroviruses and Rhinoviruses may be isolated from feces, pharyngeal swabs, salivaand nasal aspiratesand some Enteroviruses may be isolated from skin lesions, conjunctiva cerebrospinal fluid, spinal cord, brain, heartand blood.

Polio virus cultivation Is performed with the help of tissue culture technique.. The most specific of the conventional laboratory tests used to identify Picornavirus serotypes is the Neutralization test.

Serodiagnosis for the whole range of Picornaviruses is impractical because of the multiplicity of serotypes. The Neutralization test is also used to determine the immune status of a person.

8.8.8. Control

The Salk-type inactivated poliovirus vaccine (IPV) consists of a mixture of three poliovirus serotypes grown in monkey kidney cell cultures and made noninfectious by Formalin treatment. It is given in two intramuscular injections spaced a month apart The Sabin-type live attenuated Oral Poliovirus Vaccine (OPV) is commercially available as trivalent antigen. The viruses are attenuated by multiple passages in monkey kidney or human diploid cell culturesand the vaccine potency is stabilized with one Molar magnesium chloride or sucrose. This vaccine mimics wild poliovirus infections by inducing serum. In 1988, the World Health Assembly, the governing body of the World Health Organization, set the goal of global eradication of poliomyelitis by the year 2000 but the programme is extended for some more years.

Check Your Progress

8.18. Define Picorna Virus

8.19. What is Poliomyelitis.

8.20. Site by which Polio virus replication is ……..

NOTES

8.9. AIDS

8.9.1. Introduction

As the name AIDS implies it is a condition where there is a deficiency in the Body's Natural Defense mechanisms or the Immune System. It is acquired because it is not a hereditary or due to long-term use of some medicines such as those for the treatment of cancer, because of certain behavioral pattern. Syndrome is a group of symptoms. When one gets AIDS there can be wide range of symptoms all due to the bodies diminished ability to fight disease. It is important to remember that every one who has AIDS over a period of time depending upon their general health and natural defense mechanism of the body. AIDS is one of the Sexually Transmitted Disease, worldwide distributionand epidemic disease. It was first described in USA in 1981. The disease appears to have begun in Central Africa early as the 1950s. Montagnier and his colleagues first reported isolation of an etiological agent in 1983 from the Pasteur Institute, Paris.

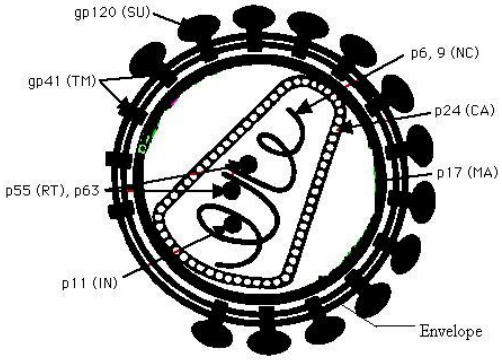
8.9.2. Prevalence Rate of AIDS

WHO estimates that 8-10 million adults & I million children worldly are infected with AIDS virus. By the year 2010 – 2025, 2 crores and 5 lakhs infected with the virus. On the basis of report 73.3% of AIDS is acquired through by Sexual contact, 7.4% AIDS acquired through Transfusion process. 0.7% through Homosexual contact, 9% through Intravenous drug abusers. Upto 1995 about 295,473 deaths have been reported in US. Mortality rate from AIDS is extremely high. The case fatality rate averages about 92% for those adults diagnosed with AIDS before 1987. By the year 2010 AIDS will become the major killer of children with an estimated 250 million infections worldwide.

The first AIDS case in India was reported in 1986 from Chennai. Since there has been rapid spread of HIV infection all over the country. By March 1998, the National AIDS Control Organization had reported that a total of 71400 people were having HIV infection from among 3.2 million people who were tested for it. Of these about 10% of AIDS cases are available only in India. About 80% were males & 20% females. Almost 89% people with AIDS were in the age group of 15-44 years, which are the most economically productive year for any individual. Maharastra has reported the maximum number of HIV infection followed by Tamilnadu & Manipur (Namakkal first in Tamil Nadu).

8.9.3. Causative Agent

Primarily the HIV-1 virus causes AIDS. This virus is a Retrovirus and closely related to Human T-cell Leukemia virus. Sometimes HIV-2 virus also causes it. HIV is an enveloped virus with cylindrical core inside. The core contains two copies of ssRNA and several enzymes. Ten virus specific proteins have been discovered. One of them the gp 120 envelope protein, participates in attachment to CD4+ cells. Virus is spherical in shape about 90-120nm in size the name HIV was given by International Committee on Virus Nomenclature in 1986.



HIV 1 virion is a pleomorphic structure containing 72 external spikes. The two major viral envelope proteins, gp 120 & gp41 form these spikes. The core of HIV 1 contains 4 nucleocapsid proteins. The phosphorylated p25 polypeptide forms the chief component of the inner shelf of the nucleocapsid, where as the p17 contains 2 copies of single stranded RNA that is associated with the various preformed virus enzymes, including Reverse Transcriptase, Integrase, Ribonuclease and protease.

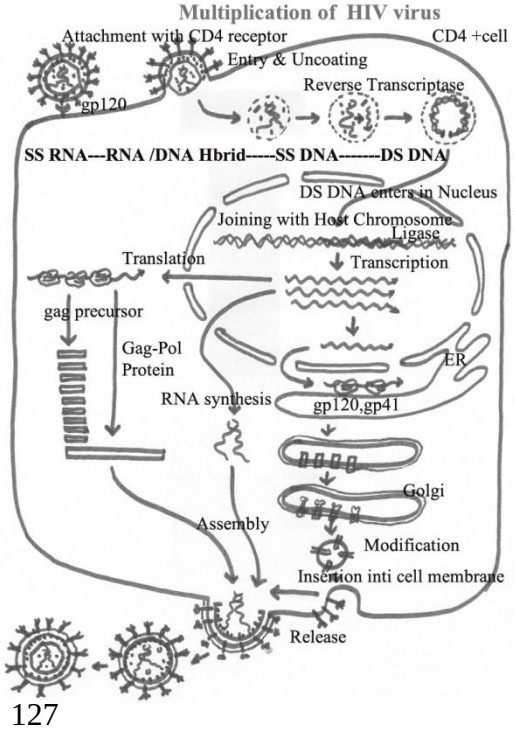
HIV is a thermolabile, being inactivated in 10 minutes at 50°C & in seconds at 100°C. At room temperature, in dried blood it may survive for upto 7 days. HIV is inactivated within 10 minutes by the treatment with 50% Ethanol, 3.5% Isopropanol, .5% Lysol, 0.5% Paraformaldehyde, 0.3% H_2O_2, 10% Household bleach. For treatment of contaminated medical instruments 2% solution of glutaraldehyde is useful.

8.9.4. Transmission

HIV is believed to have originated in Central Africa. From here it is spread to the rest of the world. HIV is primarily transmitted by Sexual contact (Homo and Hetero), Direct exposure of a person's bloodstream to body fluids, Mother to child through placenta, Skin erosion, Intravenous drug abuse, Transfusion process, Invasive medical procedure, Drug abuse with needle sharing and New born can infected through Breast breeding.

8.9.5. Pathogenesis

Once virus enters inside of the body the virus gp 120-envelope protein binds to the CD4 glycoprotein plasma membrane receptor on CD4+ T cell macrophages, dendrite cell, monocytes. After the envelope has fused with the plasma membrane, the virus releases its core protein and 2 RNA strand in the cytoplasm. Inside the infected cell, the core protein remains associated with the RNA as it is copied into single stranded DNA by the RNA/DNA dependent DNA polymerase activity of the Reverse Transcriptase enzyme,



Ribonuclease Hand the DNA is duplicated to from a dsDNA copy of the original RNA genome.

The viral dsDNA is then translocated to the nucleus and integrated into the host chromosomal DNA by the viral Integrase enzyme. This integrated viral DNA and chromosomal DNA is called **Provirus**. Then transcriptional factors stimulate transcription of proviral DNA into genomic ssRNA and after processing several mRNAs are formed. Then viral RNA is exported to cytoplasm. After completion of this process, host cell enzymes catalyses the synthesis of viral protein. HIV ssRNA and proteins assemble beneath the host cell membrane, into which gp41 and gp 120 are inserted. The cell enlarges and form bud. Bud forms a new virus. Eventually host cell lyses.

The precise mechanism of AIDS pathogenesis still is not known and many hypothesis exist. Many believe that and destruction of their function cause AIDS. Once a human's CD+4 cells are infected with HIV, 4 types of pathological changes may ensure. First, a mild form of AIDS may develop with symptoms, which include, Fever, Lymphnode enlargement, Oral Candidiasis, Presence of antibodies to HIV, Weight loss,
Malaise, Head ache. These symptoms occur in the first few months after infection, last for 1-3 weeks and recur. This is known as AIDS Related Complex (ARC).

Second symptoms appears after 2-8 years of HIV infection, although it varies considerable with each individual. Symptoms are,

Candidiasis of bronchi, trachea	Herpes simplex
Cervical cancer	Histoplasmosis
Coccodioidomycosis	Lymphoma
Diarrhoeal disease	Tuberculosis
Cytomegalovirus disease	Pneumonia
Encephalopathy	Septicemia
	Toxoplasmosis

HIV causes the third main type of disease involves the CNS, since virus infected macrophages can cross the blood brain barrier. The classical symptoms are,

Headache	Cerebrovascular disease
Fever	Brain tumor
Abnormal refluxes	Inflammation of neurons
Ataxia	Nodule formation
Auto immune neuropathies	Demyelination

The fourth result of HIV infection is cancer. Other disease are Kaposis sarcoma, Carcinoma of the mouth and rectum , B cell lymphoma

8.9.6. Laboratory diagosis

HIV infection can be detected by,

a) ***Specific test***

Virus isolation
Detection of HIV specific antibodies (ELISA)
Western blot

Polymerase chain reaction

b) ***Non specific test***

Total and Differential WBC count
Assay of T cell
Platelet count
Estimation of IgG and IgA level
Skin test for CMI

8.9.7. Prevention

Avoid sexual contact with HIV infected individuals. Don't share shaving materials. Avoid drug abuse. Follow Tamil culture. Screen blood before transfusion. Use condoms during sexual contact.

Check Your Progress

8.21. What is Provirus

8.22. What is ARC

8.23.What is CD4+ cells

8.24. Is mosquitoes are able to transmit HIV

8.10. HUMAN PAPILLOMA VIRUS

8.10.1. Introduction

Human papillomavirus (HPV) causes wart in human. According to CDC, it is a most common sexually transmitted infection. It is passed between people through skin-to-skin contact. There are over 100 varieties of HPV. This virus can affect your genitals, mouth or throat. Some cases of genital HPV infection may not cause any health problems. However, some types of HPV can lead to the development of genital warts and even cancers of the cervix, anus and throat.

8.10.2. Causative agent

HPV is a small naked, icosahedral virus with double stranded DNA as genome. It belongs to the family papova viridae. It is a non enveloped virus with 55nm diameter. It has 72 capsomeres. Papilloma viruses are a family called Papillomaviridae, which consists of a total of 170 members known as human papillomaviruses. The members are grouped into 16 genera, which are named with a Greek letter as prefix and the ending papillomavirus. For example: Alphapapillomavirus, Betapapillomavirus, etc. From a clinical point of view, the human papillomaviruses that infect the mucosa of the genital tract (which are located in genus Alphapapillomavirus) have been divided into two groups: low-risk, mainly associated with benign genital wartsand high-risk, which have a high oncogenic potential and are the causative agents of cervical cancer. All papillomaviruses associated with cancer are located in the genus Alphapapillomavirus.

8.10.3. Viral genome and proteins

Molecular weight of the DNA is 5.2 million Daltons. The papillomavirus genome is between 6800 and 8400 base pairs (bp) and is associated with host histone proteins like H2A, H2B, H3 and H4. The virus contains a non-coding regulatory region, which is called the long control region (LCR) and a region containing late expression genes, giving rise to two structural proteins. In total there are 9 or 10 open reading frames. The LCR contains response elements for cellular transcription factors such as AP1, SP1, OCT1, etc., as well as viral proteins E1 and E2, which control replication and expression of the viral genome. Reading frames are grouped into two sets called early expression genes (E) and late expression genes (L). In the first group are E1, E2, E4, E5, E6and E7, while in the second are L1 and L2. Two additional reading frames can be identified in some papillomavirus, which are designated E3 and E8.

Table8.1. Proteins of the human papillomavirus and associated functions

Type of protein	Name	Associated functions or activities
Non-structural	E1	Helicase. Essential for replication and transcription
	E2	Essential for viral replication and transcription, genomic segregationand encapsidation
	E4	Regulates late gene expression, controls viral maturation and output of virions
	E5	Stimulates the transforming activity of E6 and E7, promotes cell fusion
	E6	Binds and induces the degradation of tumor suppressor protein p53, inhibiting apoptosis; interacts with proteins of the innate immune system.
	E7	Binds and induces degradation of tumor suppressor protein pRB; increases activity of cyclin-dependent kinases; affects the expression of genes of phase S by direct interaction with E2F transcription factors and histone deacetylase; contributes to avoidance of immune response
Structural	L1	Primary capsid protein. Recognizes receptors on the host cell. Highly immunogenic and induces neutralizing antibodies
	L2	Secondary capsid protein. Participates in the union of virion to cell, entrance to celland transport to the nucleus, genome releaseand virion assembly

8.10.4. Multiplication and Pathogenesis

Papillomaviruses infects only squamous epithelial cells of skin and mucous membrane. Virus multiplied in the basal layer of the skin. The replicative cycle of papillomaviruses is commonly divided into two stages. They are early and late. The establishment of the virus in tissue requires infection of the basal keratinocytes. The introduction of virions into the cell is initiated by the interaction of L1 protein with heparan sulfate and syndecan 3 on the cellular surface. Most papillomaviruses appear to enter the cell by clathrin-dependent receptor-mediated endocytosis. The stripping of the virion and the output of the viral genome occur in the endosome. Subsequently, the L2 protein and the genome migrate to the nucleus. Once inside the nucleus, the genome is transcribed in a series of complex processes involving the presence of multiple promoters, different mRNA modification patterns (splicing)and a differentiated production of these between different cells. E1 and E2 are the first proteins to be expressed, which generates a control in the number of copies of the episomal viral genome. These proteins are maintained at 20 to 100 copies per cell. In the suprabasal layer, expression of E1, E2, E5, E6and E7 genes contributes to the maintenance of the viral genome and induces cell proliferation, increasing the number of cells able to be infected, which results in increased viral production.

Important feature usually associated with the high-risk virus is that the viral genome is integrated into the genome of the cell, while in the low-risk virus the genome remains episomal. This integration process has been associated with the move from a high-grade lesion to invasive cancer.

HPV infected cells have large perinuclear vacuoles surrounded by dense cytoplasm and form a special structure called Koilocytosis. It usually takes 3-4 months for the development of benign outgrowth of cells in to warts. Many warts are resolved spontaneously and are benign. Certain types of papilloma are associated with dysplasia that may become cancerous. HPV causes different types of warts depends on the type of virus, location of infection and effectiveness of host immune response.

8.10.5. Symptoms

Many men that are infected with HPV have no symptoms, although some may develop genital warts. Some women may notice that they have genital warts, which can appear inside the vagina, in or around the anusand on the cervix or vulva. Skin lesions are plantar wart, common wart, flat wart, epidermodysplasia verrucafermis. Mucous lesions are laryngeal papilloma, oral papilloma, conjuctival papilloma, carcinoma, condyloma, high grade dysplasia and focal epithelial hyperplasia. HPV can also cause cervical cancer and other cancers of the genitals, head, neckand throat. Some strains of HPV can cause penile, analand throat cancer in men.

8.10.6. Lab Diagnosis

HPV does not grown in conventional tissue culture methods. . histological appearance of hyperplasia of pricle cells and excess production of keratin confirms HPV infection. Testing for HPV is different in men and women. PCR amplification tests confirm HPV in both men and women. Regular Pap tests help to identify abnormal cells in women. This can signal cervical cancer or other HPV-related problems.

8.10.7. Treatments

Most cases of HPV go away spontaneously on their own, so there's no treatment required. Genital warts can be treated with antiviral drugs, burning with an electrical current or freezing with liquid nitrogen.

8.10.8. Prevention

The easiest ways to prevent HPV are to use "condoms and to practice safe sex. The Gardasil 9 vaccine is available for the prevention of genital warts and cancers caused by HPV. The vaccine can protect against nine types of HPV. The CDC recommends the HPV vaccine for boys and girls ages 11 or 12.

Check Your Progress

8.25. What is the genome of HPV.

8.26. Name the target cell of HPV.

8.27. What is Koilocytosis

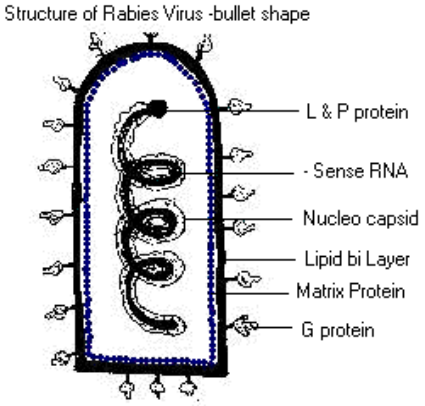
8.11. RABIES

8.11.1.Introduction

Rabies is acute, fulminant, fatal encephalitis. It is a madman disease that has instilled terror in human society. The reason is that, with rare exceptions, all of the people who are bitten by a rabid animal, got rabies. Rabies is an important zoonotic infection in which man is dead end of the infection and hence doesn't play any role in its spread to new host. In most of the developing countries, dogs are the principal reservoir of rabies (canine rabies) where as sylvatic rabies involving animal such as foxes, raccoons, cats, fats and coyotes. Rabies has been recognized from very ancient times. The word Rabies derived from the Latin word Rabidus, which means mad. It is an epidemic disease.

8.11.2. Causative Agent

Rabies virus caused rabies infection. It belongs to the family Rhabdovirus and genus Lyssa virus. In Greek Lyssa means Rabies. Rabies virus is bullet shaped
Size is about 180 x 75 nm. Genome is negative sense single stranded RNA. It is nucleo capsid in nature
Two layers, matrix layer and outer envelope cover the genome. Matrix is made up of M protein. Outer



envelope is made up of lipid bilayer as like plasma membrane. External envelope having spike like projections. It is made up of glycoproteins. Spikes are responsible for pathogenic property of the virus. RNA dependent RNA polymerase is responsible for genome replication. L and P proteins control its activity. Rabies viruses of man and animals all over the world appears to be of a single antigenic type.

Antigens of Rabies viruses are G protein, M protein, N protein, Hemaglutinin

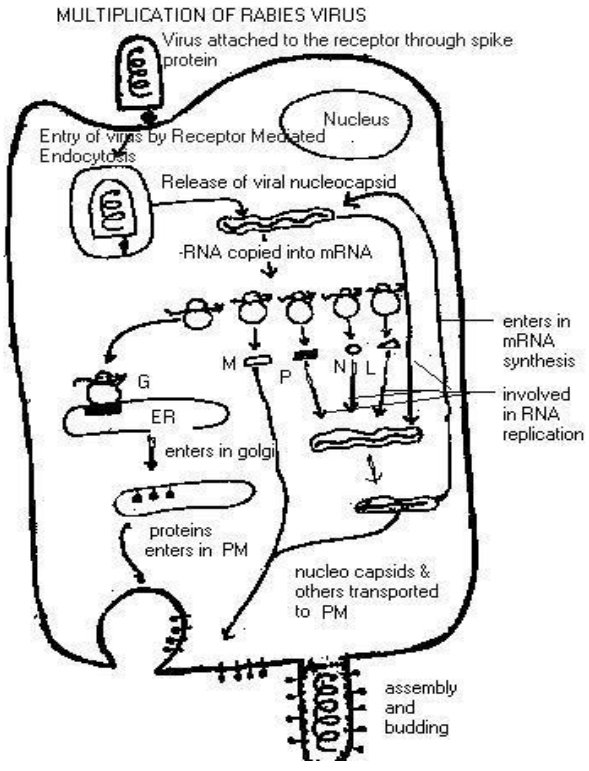
Chemical compositions of the viruses are 4% RNA, 67% protein, 26% lipid and 3% carbohydrate

Susceptibility of virus towards physical and chemical agents

Rabies virus is highly resistant against cold, dryness and decay. The virus is highly thermolabile with a half-life of approximately 4 hours at 40°C and 35 seconds at 60°C. The virus cannot withstand pH less than 4 or more than 10. It is also susceptible to oxidizing agents, most organic solvents, surface acting agents and quaternary ammonium compounds. Proteolytic enzymes, ultraviolet rays and X-rays rapidly inactivate the Rabies virus. Soaps and detergents are effective against the Rabies virus because of their lipid eliminating property, which destroys the outer covering of the virus.

8.11.3. Symptoms

Incubation period is about 30-60 days. Symptoms are Hydrophobia, Lock jaw, Encephalitis, Hysteria, Acute polyneuritis, Polio myelitis, Fever, Vomiting, Excessive salivation, Paralysis of lower jaw, Restlessness, Convolescence, Paralysis leads to death



Multiplication

Virus binds to the cellular receptor and enters the cell via receptor mediated endocytosis.

The viral membrane fuses with the membrane of the vesicle releasing viral nucleocapsid.

This structure comprises negative sense RNA coated with nucleocapsid and a small number of L and P proteins, which catalyze RNA replication.

Negative sense RNA is copied into 5 subgenomic mRNA by L and P proteins.
The N,P,M and L mRNAs are translated by free cytoplasmic mRNA
G mRNA is translated by ribosome's bound to the endoplasmic reticulum.
Newly synthesized P, N and L proteins involved in RNA replication. This process begins with positive sense RNA synthesis.
Positive sense RNA of the host serves as the template.
Some of the negative sense RNA enters to viral protein synthesis
G mRNA transcribes and synthesis glycoproteins.
G proteins travel to the plasma membrane.
Progeny nucleocapsid and M proteins are transported to the adjacent area of plasma membrane.
Assembly takes place and new viruses are released through budding process and infect new cells.

8.11.5. Pathogenesis

The virus present in the saliva of the Rabid animal is deposited in the biting site. The virus attaches cells via spikes to nicotinic acetylcholine receptors of tissue cells. The virus appears to multiply in the muscles, connective tissues or nerves at the site of deposition. It penetrates the nerve endings either immediately or after a varying interval and travels in the axoplasm towards the spinal cord and brain. The movement of the virus in the axons is passive at a speed of about 3mm per hour. The virus then multiplies extensively in brain tissue, causing the symptoms of encephalitis. Characteristic inclusion bodies called negri bodies, form at the site of viral replication in the brain but the cells are not lysed.

The virus spreads outward from the brain via the nerves to various body tissues, notably the salivary glands, eye and fatty tissue under the skin as well as heart and other vital organs.

The immune response of the host probably plays an important role in pathogenesis since viral antigens are expressed on the surface of the infected cells.

8.11.6. Labdiagnosis

Specimen - Saliva / sputum, Skin biopsy, Hair follicle, Cerebrospinal fluid, Blood, Corneal swab, Urine, Brain

Laboratory tests

Negri body examination, Fluorescent antibody test, Mouse inoculation, Serum virus neutralization test, Complement fixation test, Counter immuno electrophoresis test, Enzyme Linked Immuno Sorbent Assay, Immuno peroxidase test, Hemagglutination test, Hemagglutination inhibition test, Passive hemagglutination test, Passive diffusion, Electron microscopy, Tissue culture techniques, Polymerase Chain Reaction.

Negri body examination

Sellers staining procedure is followed. It is an Intracytoplasmic inclusion bodies. Cut and open the brain. Expose hippocampus region. Cut small piece and place it on a filter paper with cut surface facing upwards. Place the filter paper on a glass slide. Lightly sponge cut surface with the edge of filter paper to remove blood. Press clean microscopic slide on the tissue piece. While the smears are wet, flood the smear with working stain. Stain for 2-3 seconds. Wash with water. Air dry and Examine under 100 X.

Observation

Nerve cells : Blue cytoplasm and dark blue nucleus
Stroma : Pink
Erythrocytes : Copper colored
Negri bodies : Magenta to dark red with dark blue or black inner granules.

Freshly isolated strains of Rabies virus are called street virus.

8.11.7. Prevention and Treatment

This section was categorized into three, all three carry equal importance and one should not be given undue importance, or utter neglect, at the cost of other two components. These components are Management of wound, Post exposure immunization and Pre exposure immunization.

Management of wounds - Since the Rabies virus enters the human body through a bite or scratch, it is imperative to remove as much as saliva with soap. After the removal of soap, any quaternary ammonium compound (1- % cetrimonium bromide) may be applied as antiseptic along with antirabies serum.

Post exposure immunization - Because of long incubation period of rabies it is possible to institute prophylactic post exposure immunization. Immunization must be started at the earliest to ensure that the individual will be protected before the rabies virus invades central nervous system.
Two types of agents are employed to confer immunity to an individual who has been exposed to the Rabies virus: antirabies serum/ rabies immunoglobulin and anti rabies vaccine. Antirabies serum provides passive immunity in the form of readymade antirabies antibody to tide over the initial phase of infection.

Antirabies vaccines available in India

Semples Sheep Brain Vaccine
Human Diploid Cell Vaccine (HDCV)
Primary Chick Embryo Cell Vaccine (PCECV)
Purified Vero Cell Rabies Vaccine (PVRV)

Site of vaccination - The ideal site for vaccination is the anterior abdominal wall, this area offers enough space to accommodate 10 injections at 10 different sites and cause least discomfort to the patient.

Dose schedule - The 6-dose schedule spread over a period of three months was recommended. It is also called **Essen schedule** (proposed by

international conference on Rabies, held at Essen, Germany). Days of vaccination is 1st, 3rd, 7th, 14th, 30th and90th days.

Pre exposure immunization

There is no vaccines for mass pre exposure vaccination. The pre exposure prophylaxis, hence, is recommended for definite group of individuals who because of their profession or hobby are at higher risk of getting exposed to rabies virus.

8.11.8. Control of Rabies

Any strategy for control of rabies in developing countries shall have following components. They are Epidemiological surveillance, Mass vaccination, Dog population management, Community participation.

Check Your Progress

8.28. What is Street virus

8.29. What is Essen Schedule.

8.30. What is sellers staining.

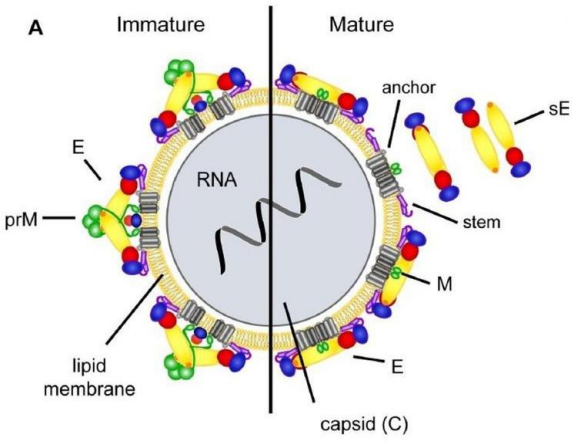
8.31. Name the vaccine used for Rabies

8.12. YELLOW FEVER

8.12.1. Introduction

Yellow fever is an acute viral haemorrhagic disease. This disease is caused by yellow fever virus. It is transmitted by Aedes mosquito infected with yellow fever virus. It causes hepatic necrosis and causing yellowing of the body hence the name yellow fever. It is an acute febrile infection.

8.12.1.Causative agent



Yellow fever virus is a prototype member of the family flaviviridae. Yellow fever is caused by yellow fever virus, flavi virus. Size of the virus is 40– to 50-nm-wide. It is a enveloped RNA Virus. It belongs to the family flaviviridae. This virus is discovered by Walter Reed in 1900. He stated that serum of infected human able to transmits this disease. It is a positive- sense, single-stranded RNA virus with 11,000 nucleotide long and has a single Open reading frame. It is a enveloped virus. Virus contains three structural proteins namely C, prM, E and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). Previousl yellow fever is called hemorrhagic fever.

8.12.3. Replication

Virus is transmitted through mosquito. After entry the viruses infects monocytes, macrophages and dentritic cells. They attach to the cell surfaces via specific receptors and are taken up by an endosomal vesicle. Inside the endosome, the decreased pH induces the fusion of of envelope with endosomal membrane, decays capsid and releases the genome. Receptor binding, as well as membrane fusion, are catalysed by the protein E.

After entering the host cell, the viral genome is replicated in the rough Endoplasmic Reticulum (ER), ie with in vesicle packets. At first, an immature form of the virus particle is produced inside the ER, whose M-protein is not yet cleaved to its mature form, so is denoted as precursor M (prM) and forms a complex with protein E. The immature particles are processed in the Golgi apparatus by the host protein furin., which cleaves prM to M. This releases E from the complex which can now take its place in the mature, infectious virion.

8.12.4. Pathogenesis

The virus is entered in to the host via the bite of Aedes mosquito. The virus multiply locally and spreads to lympnode, liver, spleen, kidney, bone marrow and myocardium. The disease is characterized by hemorrhage and circulatory collapse. Incupation period is 3-6 days. After incupation period patients experience following symptoms Histologically liver shows necrosis. This made lots of morphological change in liver cell and produce characteristic eosinophilic masses known as Councilman bodies. It is also called acidophilic intracellular inclusion Torres bodies..

8.12.5. Symptoms

Symptoms of yellow fever include fever, headache, jaundice, muscle pain, nausea, vomiting and fatigue. Complications of this disese are jaundice, albuminuria, renal failure. The patient may die due to hepatic and renal failure.

8.12.6. Lab Diagnosis

A direct confirmation can be obtained by RT PCR, where the genome of the virus is amplified. Another direct approach is the isolation of the virus and its growth in cell culture using blood plasma. This takes 1–4 weeks. Serologically, an ELSA during the acute phase of the disease using specific IgM against yellow fever. Liver biopsy can verify inflammation and necrosis of hepatocytes and detect viral antigens.

8.12.7. Prevention and Treatment

Yellow fever is prevented by an extremely effective vaccine, which is safe and affordable. A single dose of yellow fever vaccine is sufficient to confer sustained immunity and life-long protection against yellow fever disease. The vaccine provides effective immunity within 10 days for 80-100% of people vaccinated. Good supportive treatment in hospitals improves survival rates. There is currently no specific anti-viral drug for yellow fever.

NOTES

Check Your Progress

8.32. what is Councilman bodies

8.32. What is Torres Bodies

8.12. LET US SUM UP

Influenza is a viral infection of the respiratory tract cause fever, headache, muscle ache and weakness. Orthomyxo virus family member Influenza virus causes this disease. Influenza virus is transmitted from person to person primarily in droplets released by sneezing and coughing. It is highly contagious disease. The incubation period for influenza is 1-4 days. Alveoli is the primary target for virus. Infected cells will sloughs, allowing extravagation of fluid and secondary submucosal inflammation. Virus replication takes about 6 hours and kills the host cell. The virus enters permissive cells via the hemaggultinin subunit, which binds to cell membrane glycolipids or glycoprotein containing sialic acid or N-acetylneuraminic acid, the receptor for virus adsorption. The most commonly employed method for laboratory diagnosis is recovery of the virus from specimens containing respiratory secretions, such as nasal wash and throat swab or sputum. The synthetic drugs Amantadine and Rimantadine hydrochloride effectively used to prevent infection and illness caused by type A and but not by type B viruses.

Measles is a highly contagious skin disease. Measles is caused by a pleomorphic, medium sized (120-200 nm in dia) virus. It comes under Paramyxoviridae family. Its genome is RNA. It is an enveloped virus. It has two biologically active projections one 'H' is responsible for viral attachment to host cells and causes hemagglutinations. Incubation period is about 10-12 days. It begins with fever, runny nose, cough and swollen weepy eyes. Within a few days, a fine red rash appears on the fore head and spread outward over the rest of the body. Very rarely, Measles reactivation is observed after two to ten years and forms a disease called **Subacute Sclerosing Pan Encephalitis (SSPE)**, which is marked by slow progressive degeneration of the brain, resulting in death within two years.Mucous membrane involvement is responsible for an important diagnostic sign **Koplicks spot** (small bluish white ulceration on the buccal mucosa). Primary diagnosis is with the help of Kopliks spot formation. *Antigen Detection is done by* Immunofluorescence technique, immuno enzyme staining increase the sensitivity of the test.

Children too young to be vaccinated. MMR vaccine is used. Preschool children also vaccinated

Mumps results from an acute viral infection. Target of Mumps is parotid gland. Mumps begins with painful swelling of one or both parotid gland. Mumps virus causes Mumps. It is an enveloped virus included under the family Paramyxoviridae. It is a ssRNA containing negative sense virus. It is a helical shaped virus. Virus is transmitted in saliva and respiratory secretionsand its portal of entry is respiratory tract. This virus multiplies in the respiratory tract and local lymphnode in the neck. Virus

spreads throughout the body by the blood stream and produces symptoms only after infecting other tissues such as the parotid glands, meninges. Generally serological diagnosis is not necessary. This virus can be identified with hemagglutination inhibition test. Embryonated eggs and cell culture techniques are used for culturing. An effective vaccine is a variable and is often administered as part of the trivalent Measles, Mumps and Rubella (MMR) vaccine. It provides protection for at least 10 years.

Chicken pox is a common childhood infection. It is a mild, highly contagious disease, chiefly occurs in children, characterized by a generalized vesicular eruption of the skin and mucous membrane. Chicken pox is caused by Varicella virus. It belongs to herpes viridae family. Virion is spherical shaped enveloped virus. Capsid is Icosahedral in shape. Genome is linear double standard DNA. The route of infection is the mucosa of the URT or the conjunctiva. Lesions of varicella infection are associated with cutaneous and mucosal endothelial cells. Swelling of epithelial cells, ballooning degeneration and the accumulation of tissue fluids results in vesicle formation. Eosinophilic inclusion bodies are found in the nuclei of infected cells. Multinucleated giant cells are common. Incubation period is of 10-23 days. Malaise and fever are earliest symptoms. Followed by rash characteristically begins on the scalp and trunk and spreads. Macules evolve in 2-3mm vesicles, evolve in successive crops. Vidarabine and Acyclovir are useful for treatment. Zoster Immuno Globulin (ZIG) also useful.

Hepatitis is the term used for any condition where there is inflammation or necrosis of liver cells. Hetrogenous group of viruses that are called Hepatotrophic viruses causes it. These includes, Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus(HCV), Hepatitis D virus(HDV), Hepatitis E virus (HEV). Hepatitis type A is a subacute disease. It is also called infectious hepatitis. It is a 27nm nonenveloped, symmetrical (+) RNA virus. Most of its character mimics Picornovirus. HAV enters body via ingestion of contaminated food, water and fecal oral route. *Symptoms are* Fever, Malaise, Anorexia, Nausea, Vomiting, Liver tenderness. Mortality is very low ranging from 0.1-1%. General symptoms subside with the onset of Jaundice.

HBV causing Hepatitis is called Serum Hepatitis. HBV is a 42nm spherical virus that posses several antigens.There are three envelope polypeptides that come under the designation HbsAg, HbcAg & HbeAg.HBV belongs to Hepadna viridae. Incubation period varies widely from 40days-6months, but is often about 2-3 months. Discomfort in feeling, Tiredness, Fever, Diarrhoea, Jaundice, Itching sensation on skin, Inflammation in joints. The target of HBV is the hepatocyte. Virus attaches to the hepatocyte with the help of surface antigen (HbsAg). HBV enter inside of cytoplasm through receptor mediated endocytes. Replicated DNA undergoes nick formation resulting in dsDNA with staggered strand.

HCV is the leading cause of post transfusion hepatitis. Both chronic, asymptomatic carriers and chronic hepatitis have been documented with HCV. This virus is 30-60nm,spherical shaped, positive

sense RNA virus. HCV is considered as the major risk factor because 80% of infections lead to chronicity.

HDV occurs only those who have HBV infection. In 1977, Rizzetto & colleagues in Italy identify a new viral antigen in the liver cell nuclei of patients infected with HBV. Later it was called HDV.

Hepatitis E Virus is an single stranded linear RNA virus. It included under the family Calciviridae. Identified by means of ELISA. There is no vaccine currently available for preventing HEV. Personal hygiene and sanitation are the only effective way of prevention.

Polio is an ancient disease. Polio virus causes it. It is a nonenveloped virus. It is included under the family ***Picrno Viridae***. The *Picornaviruses* are (naked), small (22 to 30 nm) *icosahedral* virions *resistant to lipid solvents*. Incubation period is about 7-14 days. Primary replication of Poliovirus takes place in the oropharyngeal and intestinal mucosa (the alimentary phase). Enteroviruses and Rhinoviruses may be isolated from feces, pharyngeal swabs, salivaand nasal aspirates. The most specific of the conventional laboratory tests used to identify Picornavirus serotypes is the Neutralization test.The Salk-type inactivated poliovirus vaccine (IPV) and The Sabin-type live attenuated Oral Poliovirus Vaccine (OPV) is commercially available as trivalent antigen.

AIDS is one of the Sexually Transmitted Disease, worldwide distributionand epidemic disease. Primarily the HIV-1 virus causes AIDS. This virus is a Retrovirus. HIV is an enveloped virus with cylindrical core inside. The core contains two copies of ssRNA and several enzymes. HIV is primarily transmitted by Sexual contact (Homo and Hetero), Once virus enters inside of the body the virus gp 120-envelope protein binds to the CD4 glycoprotein plasma membrane receptor on CD4+ T cell macrophages, dendrite cell, monocytes. Many believe that and destruction of their function cause AIDS. Once a human's CD+4 cells are infected with HIV, 4 types of pathological changes may ensure. HIV infection can be detected by, Detection of HIV specific antibodies (ELISA) and confirmed by Western blot. Avoid sexual contact with HIV infected individuals. Don't share shaving materials. Avoid drug abuse. Follow Tamil culture. Screen blood before transfusion. Use condoms during sexual contact.

Human papillomavirus (HPV) causes wart in human. HPV is a small naked, icosahedral virus with double stranded DNA as genome. It belongs to the family papova viridae. Papillomaviruses infects only squamous epithelial cells of skin and mucous membrane. Virus multiplied in the basal layer of the skin. HPV infected cells have large perinuclear vacuoles surrounded by dense cytoplasm and form a special structure called Koilocytosis. Histological appearance of hyperplasia of pricle cells and excess production of keratin confirms HPV infection. The easiest ways to prevent HPV are to use "condoms and to practice safe sex. The Gardasil 9 vaccine is available for the prevention of genital warts and cancers caused by HPV.

Rabies is acute, fulminant, fatal encephalitis. Rabies virus caused rabies infection. It belongs to the family Rhabdovirus and genus Lyssa virus. In Greek Lyssa means Rabies. Rabies virus is bullet shaped. Genome is negative sense single stranded RNA. It is nucleo capsid in nature. Antigens of Rabies viruses are G protein, M protein, N protein, Hemaglutinin. Incubation period is about 30-60 days. Symptoms are Hydrophobia, Lock jaw, Encephalitis, Hysteria, Acute polyneuritis, Polio myelitis, Fever, Vomiting, Excessive salivation, Paralysis of lower jaw, Restlessness, Convolescence, Paralysis leads to death. Virus appears to multiply in the muscles, connective tissues or nerves at the site of deposition. It penetrates the nerve endings either immediately or after a varying interval and travels in the axoplasm towards the spinal cord and brain. The movement of the virus in the axons is passive at a speed of about 3mm per hour. The virus then multiplies extensively in brain tissue, causing the symptoms of encephalitis. Characteristic inclusion bodies called negri bodies, form at the site of viral replication in the brain but the cells are not lysed. Antirabies vaccines available in India

Yellow fever is an acute viral haemorrhagic disease. This disease is caused by yellow fever virus. It is transmitted by Aedes mosquito. It is a enveloped RNA Virus. It belongs to the family flaviviridae. This virus is discovered by Walter Reed in 1900. After entry the viruses infects monocytes, macrophages and dentritic cells. They attach to the cell surfaces via specific receptors and are taken up by an endosomal vesicle. Inside the endosome, the decreased pH induces the fusion of of envelope with endosomal membrane, decays capsid and releases the genome. The disease is characterized by hemorrhage and circulatory collapse. Incupation period is 3-6 days. After incubation period patients experience following symptoms. Symptoms of yellow fever include fever, headache, jaundice, muscle pain, nausea, vomiting and fatigue. A direct confirmation can be obtained by RT PCR, where the genome of the virus is amplified. Serologically, an ELSA during the acute phase of the disease using specific IgM against yellow fever. Liver biopsy can verify inflammation and necrosis of hepatocytes and detect viral antigens. Yellow fever is prevented by an extremely effective vaccine, which is safe and affordable.

8.13 UNIT END EXERCISES

Two mark Questions

Influenza A
Reys syndrome
MMR
SSPE
Inclusion bodies
Serum Hepatitis
Australian antigen
Blumberg.
Infectious Hepatitis
Jaundice
ARC
Negri Bodies

Yellow fever

Five Mark Questions

What is Koplics spot? Explain
What arethe symptoms of measles
Explain method of measles diagnosis
Mention prevention and treatment of measles.
Explain the pathogenesis of chicken pox
Differentiate Hepatitis viruses
Give a brief note of Hepatitis D Virus infection
Give a brief note on pathogenesis of HAV
Write a structure of HBV.
Explain replication of Rabies virus.
Give a brief note on HPV.
Writ about yellow fever

Ten Mark Questions

Write an essay on influenza virus.
Write a detailed note on measles
Describe Mumps.
Is chiken pox is a preventable disease? Explain.
Explain Jaundice caused by HBV
Polio is a antcient noncurable disease? Explain.
Discuss in detail about HPV.
Explain lab diagnosis of Rabies and Yellow fever.
Explain replication of HIV.

8.14. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

8.1. Orthomyxo virus is a RNA virus which cause flu in human.
8.2. Smith in 1933
8.3. Major antigenic change is called antigenic shift.
8.4. Minor antigenic change is called antigenic drift.
8.5. It is a acute encephalopathy caused by influenza A virus.
8.6. Small bluish white ulceration on buccal cavity. It is caused b measles.
8.7. Measles causing multinucleated giant cell is called Warthin Finkeldey cells.
8.8. Sub acute Spongiform Pan Encephalitis caused by measles.
8.9. Yes
8.10Mumps means Mumple, inflammation of salaivay gland.
8.11. Haemagglutinin, Neuraminidase, Fusion Protein.
8.12. Post Hepatic Neuralgia is a complication of zoster.
8.13. Cow dry inclusison – Giant cells.
8.14. Surface antigen of HBV is called Australian antigen.
8.15. Blumberg
8.16. Satellite Virus
8.17. Co-Infection means two virus enter and cause infection.
8.18. Smal RNA Viruses – Polio virus.
8.19. Poliomyelitis is a spinal cord infection individual with withered leg.
8.20. Cytoplasm

8.21. Integrated form of virus genome and host genome are called provirus.
8.22. AIDS Related Complex
8.23.TH cells are called CD4 cells.
8.24. No
8.25. Double stranded DNA
8.26. Epithelial and endothelial cells.
8.27. HPV produces large perinuclear vacuoles is called Koilocytosis.
8.28. Rabies virus is called Street virus
8.29. Rabies vaccination schedule.
8.30. Used to stain Negri bodies in Rabies cases
8.31. Semple sheep Brain Vaccine
8.32. Councilman bodies are an eosinophilic inclusion produced by Yellow fever virus.
8.32. Torres Bodies is an acidophili inclusion produced by Yellow fever virus.

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UNIT IX

Summary

9.1 INTRODUCTION

Viruses cause variety of infection. It may be transmitted through mosquito, aerosols etc. some of these viruses recently

emerged and causes high morbidity and mortality rate. In this chapter we discussed such dangerous viral diseases of human.

9.2 OBJECTIVES

After reading this chaper learners will be able to

Understsand important mosquito borne infction.

Understand severity of viaral infection.

Understand causative agent, pathogenesis, labdiagnosis of Dengu, SARS, Japenese encephalitis and Swine Flu.

NOTES

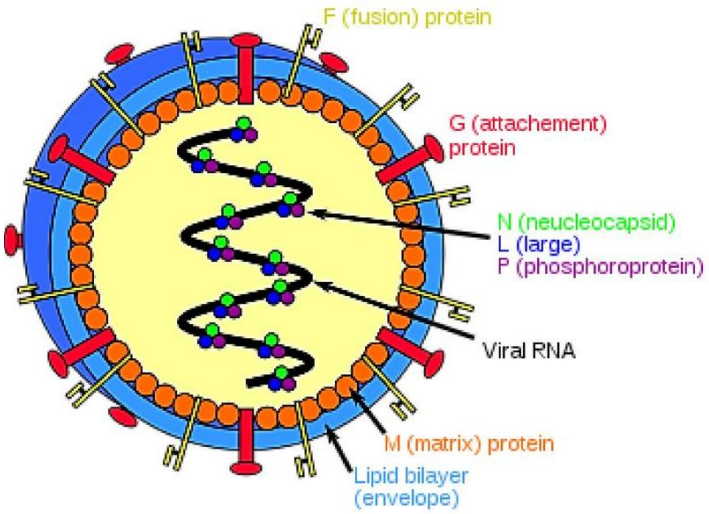
9.3. DENGUE

9.3.1. Introduction

Dengue is one of the most important human infection. It is caused by Dengue virus. This virus is included under the family Flaviviridae, genus Flavi virus. In its serious form Dengue causes two important manifestations. Dengue hemorrhagic fever and Dengue Shock Syndrome. Dengue virus is transmitted from person to person by *Aedes aegypt* mosquitoes. It is in Arbo virus group as it is transmitted through Arthropod vector Mosquito. It is also called Break bone fever.

9.3.2. Causative agent

Dengue virus causes this disease. It is an enveloped virus. This virus is closely related Yellow fever virus. It is a positive sense single-stranded RNA genome. Genome is packaged in the capsid. The outer envelope is formed by envelope protein and is the protective antigen. It aids in entry of the virus into the inside of the cell. The genome also encodes several nonstructural proteins (NS1, NS2a, NS2b, NS3, N4a, NS4b, NS5). NS1 is produced as secretory form also. NS3 is a putative helicaseand NS5 is the viral polymerase.



9.3.3. Signs and symptom

The most common symptom of dengue is **fever** with any of the following. They are Nausea, vomiting, Rash, Aches and pains (eye pain, typically behind the eyes, muscle, joint, or bone pain). Symptoms of dengue typically last 2–7 days. Most people will recover after about a week.

9.3.4. Warning signs of severe dengue

Stomach or belly pain, tenderness, Vomiting (at least 3 times in 24 hours), Bleeding from the nose or mouth, Muscle and Joint pain, rash, diarrhea, hypertension, Pleural effusion, Vomiting blood, or blood in the stool, intestinal bleeding.

9.3.5. Pathogenesis

The virus enters the blood through mosquito bite. After entry, it attachs WBC, reproduces inside the cells and move throughout the body. The white blood cells produce cytokines and interferons, which are responsible for many of the symptoms of dengue. In severe infection, the virus production inside the body is greatly increased and it affects liver and bone marrow. Fluid from the bloodstream leaks through the wall of small blood vessels into body cavities due to capillary permeability. As a result, less blood circulates in the blood vesselsand the blood pressure becomes so low that it cannot supply sufficient blood to vital organs. Furthermore, dysfunction of the bone marrow due to infection of the stromal cells leads to reduced numbers of platelets, this increases the risk of bleeding, the other major complication of dengue fever. In a small proportion of cases, the disease develops into severe dengue, also known as dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs.

9.3.6. Replication

The virus enters the blood through mosquito bite. After entry, it attachs langergans cells. The virus enters cells through mannose receptor. The dendritic cell moves to the nearest lymph node. Meanwhile, the virus genome is translated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins that replicate the viral RNA and begin to form viral particles. Immature virus particles are transported to the Golgi apparatus, the part of the cell where some of the proteins receive necessary sugar chains (glycoproteins). The now mature new viruses are released by exocytosis. They are then able to enter other white blood cells, such as monocytes and macrophages.

9.3.7. Lab Diagnosis

The earliest change detectable on laboratory investigations is a low white blood cell count, which may then be followed by low platelets. Elevated levels of SGOT and SGPT along with low platelet count indicate dengue. In severe disease, plasma leakage results in hemoconcentration and hypoalbuminemia. Diagnosis can be done by virus isolation in cell cultures, nucleic acid detection by PCR, viral antigen detection (such as for NS1) or specific antibodies(serology).

9.3.8. Prevention

The primary method of controlling A. aegypti is by eliminating its habitats. This is done by getting rid of open sources of water, Generalized spraying of organophosphae insecticides. People can

prevent mosquito bites by wearing clothing that fully covers the skin, using mosquito netting while restingand/or the application of insect repellent (DEET being the most effective). The vaccine is produced by Sanofi and goes by the brand name Dengvaxia. It is based on a weakened combination of the yellow fever virus and each of the four dengue serotypes.

9.3.9. Treatment

There is no specific medication to treat dengue. Treat the symptoms of dengue and see your healthcare provider. **Rest** as much as possible. **Take acetaminophen or** paracetamol to control fever and relieve pain. Do not take aspirin or ibuprofen. **Drink plenty of fluids** such as water or drinks with added electrolytes to stay hydrated.

Check your Progress

9.1. What is the major symptom of dengue

9.2. Which lab test indicate dengue infection

9.3. why DEET being more effective in dengue

9.4. JAPANESE ENCEPHALITIS

9.4.1. Introduction

Encephalitis means inflammation of the brain. Japanese encephalitis (JEV) is an infection of the brain. It is caused by the Japanese encephalitis Virus (JEV). This virus is transmitted through mosquito culex. Pis and wild birs are reservoir for this virus.

9.4.2. Causative agent

It is a disease caused by Japanese encephalitis virus (JEV) transmitted by Culex mosquito. This virus belongs to the family flaviviridae, geneus flavivirus and species Japanese encephalitis Virus. It is an enveloped virus. This virus is closely related to the West Nile virus and the St. Louis encephalitis virus. It is a positive sense single-stranded RNA genome. Genome is packaged in the capsid. The outer envelope is formed by envelope protein and is the protective antigen. It aids in entry of the virus into the inside of the cell. The genome also encodes several nonstructural proteins (NS1, NS2a, NS2b, NS3, N4a, NS4b, NS5). NS1 is produced as secretory form also. NS3 is a putative helicaseand NS5 is the viral polymerase.

9.4.3. Pathogenesis

Pathogenesis of Encephalitis is not well studied. The virus enters in the human body through mosquito or tick bite. After entry the virus multiplies in non-neural tissue and is present in the blood 3 days before first sign of involvement of the CNS. Then the virus multiplies in the brain cells, destroyed the cell and encephalitis become apparent.

Mortality of this disease varies but is generally higher in children. Transplacental spread has been noted. Lifelong neurological defects such

as deafness, emotional lability and hemiparesis may occur in those who have had central nervous system involvement.

Increased microglial activation following Japanese Encephalitis infection has been found to influence the outcome of viral pathogenesis. Microglia are the resident immune cells of the central nervous system (CNS) and have a critical role in host defense against invading microorganisms. Activated microglia secrete cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α), which can cause toxic effects in the brain. Additionally, other soluble factors such as neurotoxins, excitatory neurotransmitters, prostaglandin, reactive oxygenand nitrogen species are secreted by activated microglia.

Although the net effect of the proinflammatory mediators is to kill infectious organisms and infected cells as well as to stimulate the production of molecules that amplify the mounting response to damage, it is also evident that in a nonregenerating organ such as the brain, a dysregulated innate immune response would be deleterious. In JE the tight regulation of microglial activation appears to be disturbed, resulting in an autotoxic loop of microglial activation that possibly leads to bystander neuronal damage. In animals, key signs include infertility and abortion in pigs, neurological disease in horses and systemic signs including fever, lethargy and anorexia.

9.4.4. Replication

Virus is attached to host receptor through envelope protein E. this is mediated by clathrin. This prcess is called receptor mediated endocytosis. Virus has tissue tropism with skin epithelium, kidney epithelium, intestinal epithelium and testes epithelium. Flaviviruses have a (+) sense RNA genome and replicate in the cytoplasm of the host cells. The genome mimics the cellular mRNA molecule in all aspects except for the absence of the poly-adenylated (poly-A) tail. This feature allows the virus to exploit cellular apparatus to synthesise both structural and non-structural proteins, during replication. The cellular ribosome is crucial to the replication of the flavivirus, as it translates the RNA, in a similar fashion to cellular mRNA, resulting in the synthesis of a single polyprotein. In general, the genome encodes 3 structural proteins (Capsid, prMand Envelope) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The genomic RNA is modified at the 5′ end of positive-strand genomic RNA with a cap-1 structure (me7-GpppA-me2).

Cellular RNA cap structures are formed via the action of an RNA triphosphatase, with guanylyltransferase, N7-methyltransferase and 2′-O methyltransferase. The virus encodes these activities in its non-structural proteins. The NS3 protein encodes a RNA triphosphatase within its helicase domain. It uses the helicase ATP hydrolysis site to remove the γ-phosphate from the 5′ end of the RNA. The N-terminal domain of the non-structural protein 5 (NS5) has both the N7-methyltransferase and guanylyltransferase activities necessary for forming mature RNA cap structures. RNA binding affinity is reduced by

the presence of ATP or GTP and enhanced by S-adenosyl methionine.[9] This protein also encodes a 2′-O methyltransferase.

Once translated, the polyprotein is cleaved by a combination of viral and host proteases to release mature polypeptide products. This polyproteins are enzymatically cleave the remaining polyprotein into the individual proteins. One of the products cleaved is a polymerase, responsible for the synthesis of a (-) sense RNA molecule. Consequently, this molecule acts as the template for the synthesis of the genomic progeny RNA.

Flavivirus genomic RNA replication occurs on rough endoplasmic reticulum membranes in membranous compartments. New viral particles are subsequently assembled. This occurs during the budding process which is also responsible for the accumulation of the envelope and cell lysis.

9.4.5. Symptoms

The Japanese encephalitis virus (JEV) has an incubation period of 2 to 26 days. Fever, headache and malaise are other non-specific symptoms of this disease which may last for a period of between 1 and 6 days. Signs which develop during the acute encephalitic stage include neck rigidity, cachexia, hemiparesis, convulsions and a raised body temperature between 38–41 °C (100.4–105.8 °F). Mental retardation is usually developed.

9.4.6. Lab Diagnosis

Recovery of virus: It is by the inoculation of serum with intracerebral inoculation of suckling mice. For some virus cell lines are developed.

Serology: Neutralizing hemagglutination-inhibiting antibodies are detected within few days. CF antibodies appear later. WHO recommends testing for JEV-specific IgM antibody in a single sample of cerebrospinal fluid (CSF) or serum, using an IgM-capture ELISA. Testing of CSF sample is preferred to reduce false-positivity rates from previous infection or vaccination

9.4.7. Treatment

There is no specific treatment. Treatment is supportive to relieve symptoms and stabilize the patient.

9.4.8. Prevention and control

Safe and effective JE vaccines are available to prevent disease. WHO recommends having strong JE prevention and control activities, including JE immunization in all regions where the disease is a recognized public health priority, along with strengthening surveillance and reporting mechanisms.

Check your Progress

9.4. Mental retardation is the major indication of JF? Why.

9.5. Mention the mode of release of JE Virus

NOTES

9.5. SARS

9.5.1. Introduction

Severe acute respiratory syndrome or SARS is a contagious and potentially fatal respiratory illness. It first appeared in China in November 2002 and was identified in February 2003. SARS spread to over 24 countries before it was contained.

9.5.2. Causative agent

The SARS is caused by coronavirus (SARS-CoV). A coronavirus is a common form of virus that typically causes upper-respiratory tract illnesses. The common cold results from a kind of coronavirus. Six different kinds of coronavirus are known to infect humans. Four of these are common and most people will experience at least one of them at some time in their life. The two other types cause SARS and Middle East Respiratory Syndrome (MERS).

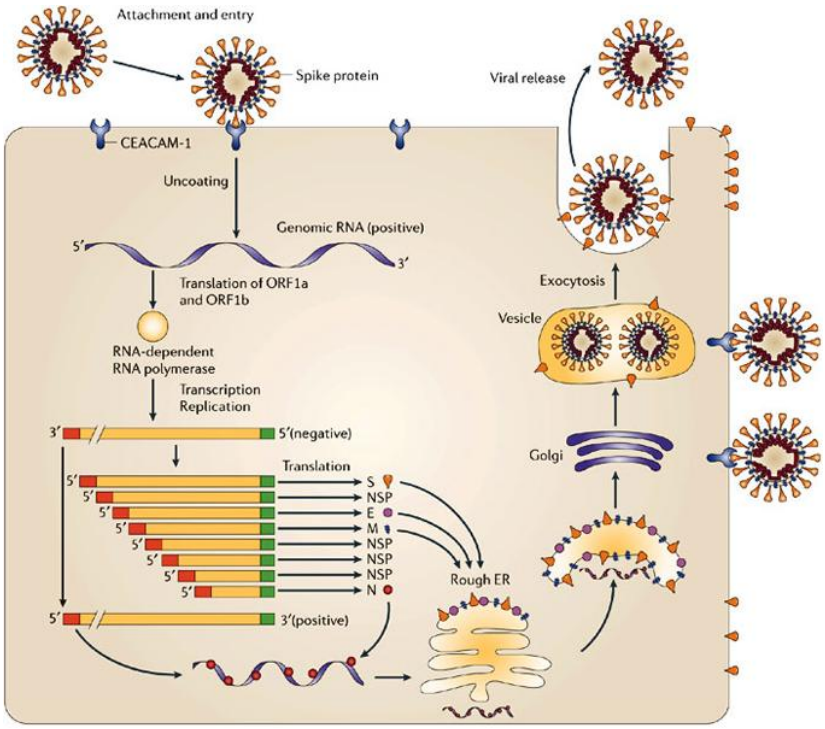
Corona viruses are belongs to Corona viridae. They are large virus. Size of the virus is 100-200nm diameter. It is a enveloped, spherical, single stranded RNA virus. Club shaped spikes are present in the envelope.

9.5.3. Transmission

Droplets from coughing and sneezing and close human contact likely transmit the SARS virus. It is also transmitted through hugging and kissing; sharing utensils for eating and drinking; speaking to someone within a distance of 3 feet; touching someone directly. The virus is likely to remain active in the environment for several days.

9.5.4. Replication

The coronavirus genomic RNA encodes structural proteins as well as non structural proteins. nonstructural proteins are responsible for viral RNA synthesis. One nonstructural protein 2 (nsp2)and one structural protein, the nucleocapsid protein (N), are involved in viral RNA synthesis.



Corona virus binds to the host-cell receptor CEACAM-1 through interaction of the spike (S) glycoprotein. Virus entry into the host cell can occur through fusion with the surface of the host cell, with the subsequent release of the genomic RNA into the cytoplasm. Translation of the positive-strand genomic RNA gives rise to a large polyprotein that undergoes proteolytic processing to generate an RNA-dependent RNA polymerase. Through the action of the RNA polymerase, a full-length, antisense negative-strand template is generated. Subgenomic mRNAs are synthesized, presumably from subgenomic negative-strand templates. Translation of subgenomic mRNAs gives rise to structural viral proteins. S glycoprotein is expressed on the surface of the host cell and this might contribute to fusion with neighbouring uninfected cells by binding to CEACAM-1. Virus assembly occurs within vesicles, followed by virus release by fusion of virion-containing vesicles with the plasma membrane. Released virus can infect other cells and can replicate within the parent cell through binding to CEACAM-1. E, envelope protein; ER, endoplasmic reticulum; M, membrane protein; N, nucleocapsid protein; ORF, open reading frame.

9.5.5. Pathogenesis

Coronaviruses primarily infect the upper respiratory and gastrointestinal tract of mammals and birds. Six different currently known strains of coronaviruses infect humans. Coronaviruses are believed to cause a significant percentage of all common colds in human adults and children. Coronaviruses can cause pneumonia.

9.5.6. Symptoms

Incubation period is 2 to 7 days. Most cases of SARS begin with a high fever. Other early symptoms include those common to flu, such as aches, chills, diarrhea, dry coughingand shortness of breath. These will

develop over the course of a week. Serious complications, such as respiratory failure, heart failureand liver failure may occur.

9.5.7. Lab Diagnosis

The World Health Organization (WHO) advised that, a fever of at least 100.4° Fahrenheit or 38° Celsius with a cough, difficulty breathing, shortness of breath suggest a diagnosis of SARS. **Reverse transcription-polymerase chain reaction (RT-PCR)** testing can detect the virus in blood, stooland nasal secretions.

Serologic testing can detect SARS-CoV antibodies in the blood ELISA.

Viruses are cultured with Vero monkey tissue culture cells. Cytopathic effect is noted within a day.

9.5.8. Prevention

There is no specific treatment. Some simple steps can be taken to help prevent the SARS virus from spreading. Good personal hygiene practices can help restrict the spread of the virus. They are frequent handwashing, avoiding touching the eyes, mouth or nose with unclean hands, covering the mouth and nose with a tissue when coughing or sneezing.

Check your Progress

9.6. What is SARS

9.7. What is MERS

9.6. SWINE FLU (H1N1 & H3N2 INFLUENZA VIRUS)

9.6.1. Introducton

It is a highly contagious acute respiratory disease of pigs caused by type A influenza virus that regularly causes outbreaks of influenza in pigs. People with H1N1 flu virus infection should be considered potentially contagious one day before the onset of symptoms and as long as they are symptomaticand possibly up to 7 days following the onset of illness. Children, especially younger children, might be contagious for longer periods.

9.6.2. Causative agent

Mutant strin of Influenza Virus causes swine flu. H1N1 is related to the strain variation in Influenza virus. Influena viruses may undergo antigenic shift and antigenic drift mechanisms, which leads to major or minor change in the nature of spike protein like Haemagglutinin spike (H) and Neuaminidase spike (N). The changes are indicated as H1N1, H2N2, H1N2, H3N2. These changes modify the virulent property as well as immunological properties of viruses there by severity of the infection also varied.

Structure – Refer Influenza. Page No 107

9.6.3. Transmission

Swine flu viruses do not normally infect humans. However, sporadic human infections with swine flu have occurred. Most commonly, these cases occur in people with direct exposure to pigs. However, there have been cases of human-to-human spread of swine flu.

NOTES

9.6.4. Symptoms

The symptoms of H1N1 flu in people are similar to the symptoms of regular human seasonal influenza and include fever, coughing, stuffy nose and body aches. Some people with H1N1 flu have also reported having a sore throat, nausea, vomitingand diarrhea. Chest discomfort is severe in the case of swine flu, which is not indicated in common influenza. H1N1 flu in people can vary in severity from mild to severe. Swine flu infectionand H1N1 infection, can be very serious, causing complications such as pneumonia.

9.6.5. Replication

Refer Influenza Page No 107

9.6.6. Pathogenesis

Refer Influenza - Page No 107

9.6.7. Treatment

The Centers for Disease Control and Prevention (CDC) recommends the use of Four antiviral agents such as Zanamivir (Relenza), Oseltamivir (Tamiflu), peramivir (Rapivab)and baloxavir marboxil (Xofluza), for the treatment and/or prevention of infection with H1N1 flu.

Check your Progress

9.8. What is antigenic shift

9.9. What is antigenic drift

9.10. Why the name swine flu is given

9.7. LET US SUM UP

Dengue is one of the most important human infection. It is caused by Dengue virus. This virus is included under the family Flaviviridae, It is a positive sense single-stranded RNA genome. Genome is packaged in the capsid. The most common symptom of dengue is **fever** with any of the following. They are Nausea, vomiting, Rash, Aches and pains (eye pain, typically behind the eyes, muscle, joint, or bone pain). Symptoms of dengue typically last 2–7 days. Most people will recover after about a week. The virus enters the blood through mosquito bite. After entry, it attachs WBC, reproduces inside the cells and move throughout the body. The white blood cells produce cytokines and interferons, which are responsible for many of the symptoms of dengue. The earliest change detectable on laboratory investigations is a low white blood cell count, which may then be followed by low platelets. Elevated levels of SGOT and SGPT along with low platelet count indicate dengue. The primary method of controlling A. aegypti is by eliminating its habitats. There is no specific medication to treat dengue.

Encephalitis means inflammation of the brain. Japanese encephalitis (JEV) is an infection of the brain. It is caused by the Japanese encephalitis Virus (JEV). This virus is transmitted through mosquito culex. Pathogenesis of Encephalitis is not well studied. The

virus enters in the human body through mosquito or tick bite. After entry the virus multiplies in non-neural tissue and is present in the blood 3 days before first sign of involvement of the CNS. Then the virus multiplies in the brain cells, destroyed the cell and encephalitis become apparent.

The Japanese encephalitis virus (JEV) has an incubation period of 2 to 26 days. Fever, headache and malaise are other non-specific symptoms of this disease which may last for a period of between 1 and 6 days. Neutralizing hemagglutination-inhibiting antibodies are detected within few days. CF antibodies appear later. There is no specific treatment. Treatment is supportive to relieve symptoms and stabilize the patient.Safe and effective JE vaccines are available to prevent disease.

SARS is a contagious and potentially fatal respiratory illness. The SARS is caused by coronavirus (SARS-CoV). A coronavirus is a common form of virus that typically causes upper-respiratory tract illnesses. Corona viruses are belongs to Corona viridae. They are large virus. Size of the virus is 100-200nm diameter. It is a enveloped, spherical, single stranded RNA virus. Club shaped spikes are present in the envelope. Droplets from coughing and sneezing and close human contact likely transmit the SARS virus. Coronaviruses primarily infect the upper respiratory and gastrointestinal tract of mammals and birds. Coronaviruses can cause pneumonia. Incubation period is 2 to 7 days. Most cases of SARS begin with a high fever. Serious complications, such as respiratory failure, heart failureand liver failure may occur. The World Health Organization (WHO) advised that, a fever of at least 100.4° Fahrenheit with a cough, difficulty breathing, shortness of breath suggest a diagnosis of SARS. RT-PCR testing can detect the virus in blood, stooland nasal secretions.There is no specific treatment. Some simple steps can be taken to help prevent the SARS virus from spreading. Good personal hygiene practices can help restrict the spread of the virus.

H1N1 influenza is also called swine flu. It is a highly contagious acute respiratory disease of pigs caused by type A influenza virus that regularly causes outbreaks of influenza in pigs. The symptoms of H1N1 flu in people are similar to the symptoms of regular human seasonal influenza and include fever, coughing, stuffy nose and body aches. H1N1 flu in people can vary in severity from mild to severe. Swine flu infectionand H1N1 infection, can be very serious, causing complications such as pneumonia. The Centers for Disease Control and Prevention (CDC) recommends the use of Four antiviral agents such as Zanamivir (Relenza), Oseltamivir (Tamiflu), peramivir (Rapivab)and baloxavir marboxil (Xofluza), for the treatment and/or prevention of infection with H1N1 flu.

9.8. UNIT END EXERCISES

Two Mark Questions

What is hemorrhagic fever
What is Break bone fever
What is H1N1
Name few symptoms of Japanese encephalitis
What is Tamiflue

What is Aedes aegeypti

Five Mark Questions

Give the symptoms and complications of dengu .
Describe replication of denguvirus.
Explain replication and pathogenesis of Corona virus.
Give a short account on Swine flue

Ten Mark Questions

Write an essay on Japenese encephalitis
Write a note on dengue.
Describe SARS

9.9. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

9.1. Fever
9.2. Platelet count
9.3. DEET spray will reduce the Burden of mosquito bite.
.4. Yes, because it affect brain cells.
9.5. Budding
9.6. Severe Acute respiratory Syndrome
9.7. Middle East Respiratory Syndrome
9.8. Major antigenic change
9.9. Minor antigenic shift
9.10. Swine flue is transmitted from pig like group animals

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NOTES

UNIT - X FUNGAL DISEASES

Summary

10.1 INTRODUCTION

10.1.1. General Characters

Fungi are Eukaryotic organisms. Each Fungal cell has Nucleus, Nuclear Membrane, Endoplasmic Reticulum and Mitochondria. Mycology is the study of Fungi. Fungi are aerobic or facultatively anaerobic chemoheterotrophs. Fungi synthesize lysine from α- aminoadipic acid. Many Fungal species produce flagellated, motile cells. Fungi lack the property of photosynthesis. They are found as saprophytes in the soil, they have the capability to degrade the organic matter.

10.1.2. *Morphology*

There are two forms of fungi that are Yeast and Molds. Some times yeast are looks like mould due to the formation of pseudohyphae.

Yeast : Yeast is round or oval shaped, 4-5μm in diameter. Some yeast is 24μm in diameter. On SDA agar, yeast produce creamy opaque colonies. Eg: *Cryptococcus neoformans*

Yeast like fungi : Some yeasts produce false hyphae during some time called pseudohyphae. These yeasts are called yeast like fungi. Eg. *Candida albicans*

Molds : Multicellular fungi composed of filamentous or tubular structures called hyphae. The length of single hyphae is about 5-50μm. Some hyphae have the cross walls called Septa. Hyphae are branched and

form a mat like structure is called as Mycelium. On SDA agar medium molds produce cottony colonies.

10.1.3. *Fungal Cell Wall*

Cell wall is rigid and thick, 15% - 30% of the dry weight of fungal cell wall contains some essential components such as polysaccharides and proteins. It provides rigidity, strength and it protects the cell membrane form osmotic shock.

Cell wall of yeast thickness is about 200-300nm in diameter and molds wall thickness is about 200nm in diameter.

10.1.4. Reproduction

Fungi reproduced by asexually and sexually, asexual reproduction includes Fragmentation, Fission, Budding and Spore formation. Sexual reproduction includes Oospores, Zygospores, Ascospore and Basidiospores formation. The spores derived from sexual means are called Telomorphic and spores that are produced by asexual means are called Anamorphic. Based on Spores and Conidia fungus are categorized. Some important Conidia are

Arthroconidia (Arthrospores)
Blastoconidia (Blastospores)
Chlamydoconidia (Chlamydospores)
Phialoconidia (Phialospores)
Sporangioconidia (Sporangiospores)

10.1.5. Medically Important Divisions of Fungi

Deuteromycota
Zygomycota
Ascomycota
Basidiomycota.

10.1.6. Fungal Diseases

Superficial Mycoses (Tinea versicolor)
Cutaneous Mycoses (Tinea capitis)
Systemic Mycoses (Histoplasmosis)
Opportunistic Mycoses (Candidiasis)

10.1.7. *Economic importance of fungi*

Saccharomyces and Trichoderma are used in the production of foods. They are used for the biological control of pests. Many fungal members cause spoilage of fruits, grains and vegetables and also cause plant diseases. Some of the fungus are used as a food (Mushroom)

10.1.8. Superficial Mycoses

Superficial mycoses affect the skin, hair or nail. These infections are mild but sometimes chronic in nature. Those fungi that cause superficial infection have the capability to digest keratin and are saprophytes. It induces less inflammatory responses. There are two types of superficial infections, Surface Mycoses and Cutaneous Mycoses.

Eg. Pityriasis versicolor (Tinea versicolor), Piedra (Trichosporosis)

10.1.9. Cutaneous Mycoses

Cutaneous Mycoses are observed slightly deeper portion of the Epidermis. In this case, the fungi grown in the cornified layer of the skin and cause inflammatory response. Eg. *Dermatophytosis - Microsporum sp., Trichophyton sp., Epidermophyton spp.*

10.1.10. Subcutaneous Mycoses

These are a group of fungus involve in the Dermis and Subcutaneous tissue. These are referred as ***Mycoses of implantation*** because they are acquired when the pathogen is inoculated through the skin by minor cuts or scratches or by thorns or splinter wound. Etiological agents are ubiquitous in nature and usually found in soil or on decaying vegetation. The infections occur on the parts of the body that are most prone to be traumatized E.g. Feet, Legsand Buttocks. Subcutaneous infections are slow in onset and lesions evolve over many months. Persistence may be due to noninvasive properties of this group of organisms.

Infections are, ***Chromomycosis, Mycetoma***

10.1.11. Systemic Mycoses

These are also called deep mycoses. They are acquired by inhalation. There are two types of systemic mycoses, that are Primary Systemic Mycoses and Opportunistic Systemic Mycoses. Those fungi that cause primary systemic mycoses are called primary pathogen.

Eg. *Blastomycosis, Paracoccidioidomycosis, Coccidioidomycosis Histoplasmosis*

Some of the saprophytic fungi of the environment cause Opportunistic Systemic Mycoses and are called Opportunistic Fungi.

Eg. *Cryptococcosis, Candidiosis*

10.2 OBJECTIVES

After reading this chapter learner will be able to understand

Fungal infections of human
Reason for fungal diseases
Prevention of fungal disease
Treatment of fungal diseases

10.3. SUPERFICIAL MYCOSIS

10.3.1. Pityriasis Versicolor

10.3.1.1.Introduction

It is also called ***Tinea versicolor***. It is a chronic and less serious infection of the skin. This infection involves **stratum corneum** characterized by discoloration or depigmentation of skin. The organism is found world wide as a commensal on smooth skin of humans.

10.3.1.2. Causative agent

It is caused by *Malassezia furfur*. It is a superficial skin infection. Malassezia is a name of mycelial form. Etiological agent is a dimorphic fungus. It infects only skin. It is a normal flora of the human skin. Chest,

abdomen, upper limps and back are the susceptible area to *M.furfur*. There are two types of yeasts oval yeasts included under serovar C *(Pityrosporum ovale)* and round yeasts having two serovars A and B *(Pityrosporum orbiculariae).*

10.3.1.3. Pathogenecity

It affects external appearance of an individual. It produces non-inflammatory macular lesions with fine scaling. Lesions are not usually itchy. Mycelial phase fungus is associated with Tinea versicolor. Fungus interferes melanin production by the production of **Dicarbolic acid**. Fungus inhibits the activity of ***Tyrosinase,*** which is responsible for the synthesis of melanin. Some individuals develop folliculitis.

10.3.1.4. Lab Diagnosis

Specimen **-** Pus skin scrapings

Microscopy **-** Direct microscopic observation of the specimen with 10-20% KOH. Short unbranched hyphae and spherical cells are observed. Lesions also fluorescence under woods lamp test .

Culture **-** Modified SDA supplemented with olive oil with antibiotics is used for cultivation. *M.furfur* is a lipophilic yeast. *S*hiny or pasty white to cream coloured colonies are formed after 1-2 weeks. *T*he phialoconidia are thick walled, round or oval in shape. They typically occur in clusters. Individual cells looks like yeast.

10.3.1.5. Epidemiology

Incidence increases where the climate is hot and humid and is highest in trophics. Its occurrence has been related to the presence of certain aminoacid and hydrophobic compounds on the skin.

10.3.1.6. Treatment

It is by the application of *Selinum sulfide*

10.3.2. Tinea Nigra

10.3.2.1. Introduction

It is a superficial, chronic and asymptomatic infection of the Stratum Corneum caused by the dematiaceous yeast

10.3.2.2. Causative agent

It is caused by Exophiala werneckii. It is is one of the saprophytic fungi. Dimorphism is observed in cultures. Fungus is ubiquitous in nature. During aging mycelial forms are converted into yeast form. Colony is shiny, moist and often white to gray in color initially. Within a few days, the colony darkens and becomes olive to black. Later, mycelium will develops and the colony appears dull and fuzzy.

10.3.2.3.Clinical Condition

Most frequently organisms found in tropical areas. Approximately 95% cases occur in teen-agers. Lesions usually consist of a solitary, innocuous macuole with sharply defined margins. The brownish colour of the lesion is darkest at the advanced periphery, where

most of the actively growing organisms are located.Many cases involves the palm, other parts of the skin may also be infected, including fingers and face. The lesions resemble faded silver nitrate stain.

10.3.2.4. Lab Diagnosis

Sample - Skin scrapings and lesions

Microscopy -Specimens are mounted by 20% KOH or Calcofluor white stain.Observation reveals brown pigmented septate hyphae and budding yeast cells.

Culture-Culture with Sabuourds Dextrose Agar with or without antibiotics should recover the organism.

10.3.2.5. Treatment

It responds well to topical keratolytic solutions of sulfur, salicylic acid or tincture of iodine.

10.3.3. Piedra

There are two types of Piedra. Black piedra and white piedra

10.3.3.1. **Black Piedra**

10.3.3.1.1. Causative agent

Black Piedra is a Nodular Infection of the Hair shaft caused by *Piedraia hortae***.** The organism is a plant parasite. Human infection caused by this parasite is called black piedra. Color of the colony on SDA is greenish black or black. Asci and ascospores are rarely seen in culture. Black piedra consist of slow growing brown to reddish black mycelia. It produces spindle shaped ascospores**.**

10.3.3.1.2. Clinical Condition

Hard nodules found along with the infected hair shaft. Nodules have a hard carbonaceous consistency**.**

10.3.3.1.3. LabDiagnosis

Hair is subjected to diagnosis. SDA medium is used for cultivation. Woods lamp test also performed.

10.3.3.2. **White Piedra**

10.3.3.2.1. Causative agent

Trichosporan beigelii is a causative agent. It present as larger, softer, yellowish nodules on the hair. *Trichosporan beigelii* is sensitive to Cycloheximide. Culture is dimorphic one. Yeast cultures are white and have a pasty consistency. As the culture ages, colonies develop deep radiative farrows and take on a yellowish coloration with the creamy texture. Microscopic examination reveals septate hyphae that fragments rapidly to form arthroconidia. The Arthroconidia rapidly roundup and many cells form Blastoconidia. It affects hairs of the scalp, mustache and beard. It is characterized by the development of cream colored soft pasty growths along infected hair shaft. Piedra is endemic in tropical underdeveloped countries.

10.3.3.2.2. Lab Diagnosis

Sample - Infected hair

Microscopy - KOH mount and Woods lamp test
Culture - Growth is dimorphic, Specimen is inoculates on SDA medium.

10.3.3.2.3. Treatment

It is accomplished by the topical use of kerotolytic agents, preparation containing Selenium disulphide, Hyposulfite, Thiosulphite of salicylic acid. Miconazole nitrate is also used. Shaving or Cropping of infected hairs close to the scalp surface achieves effective therapy.

Check Your Progress

10.1. How Malassezia furfur cause discolouration of skin

10.2. What is selenium sulfate

10.3. What is ring worm infection

10.4. CUTANEOUS MYCOSES

10.4.1. Dermatophytosis

10.4.1.1. Introduction

Dermatophytosis is fungal infections that involve the superficial areas of the body. These diseases are caused by the dermatophytes that invade the keratinized portion of the hair, skin and nails. Dermatophytes are transmitted by close contact and the organisms may spread rapidly within families and enclosed communities. The dermatophytes can be conveniently classified into three groups. On the basis of their reservoir and host preference. Anthropophilic species are those which have man as their major host. The Zoophilic pathogens infect animals. Geophilic organisms are found in soil but may infect animals and man.

10.4.1.2. Causative agent

On the basis of Clinical, Morphological and Microscopic characteristics, three different genera are recognized as dermatophytes

Epidermophyton, Microsporum and Trichophyton.

10.4.1.3. Clinical Importance

The superficial cutaneous infection caused by dermatophytes is called Tinea or Ringworm infection. The lesions of the Tineas are often appear as pink circular lesions and gradually advance to form new borders. Scaling and peeling is common in affected areas. The lesions are rarely painful but can be very itchy. Anthropophilic organisms produce more intense inflammatory reactions with pustular lesions or a large inflammatory mass known as kerion. Infection of the nail is chronic and produces discoloration and thickening. Scalp infection leads to scaling and inflammation with hair lose which may sometimes associated with scarring. Dermatophyte infections are clinically classifies by anatomical location.

10.4.1.4. Pathogenesis

These fungi invade only dead corinified layers of the skin, hair and nails. Only a few organisms are necessary to induce an infection following trauma. Dermatophytes grow in filamentous form within the stratum corneum. The downward extension of this hyphae is restricted

because certain nutrients, principally iron, are not available in the deeper tissues. Consequently, lateral invasions from the focus of inoculation occur, leading to the annular pattern seen on the skin surface.

The dermatophytes do not directly damage the skin, but a delayed hypersensitivity reactions appears at the borders of the growing fungi after the host becomes sensitized to soluble fungal antigens. The inflammatory response results in a circinate pattern of erythema and oedema followed by exudation.

Invasion of hair follicle produces inflamed nodules, deep seated pustules and abscesses. The skin become inflamed only after start of cell mediated immunity to the dermatophytes.

There are two types of Dermatophytic infections

1) The acute or inflammatory type of infection(associated with CMI to the fungus, which generally heals spontaneously or responds readily to treatment)

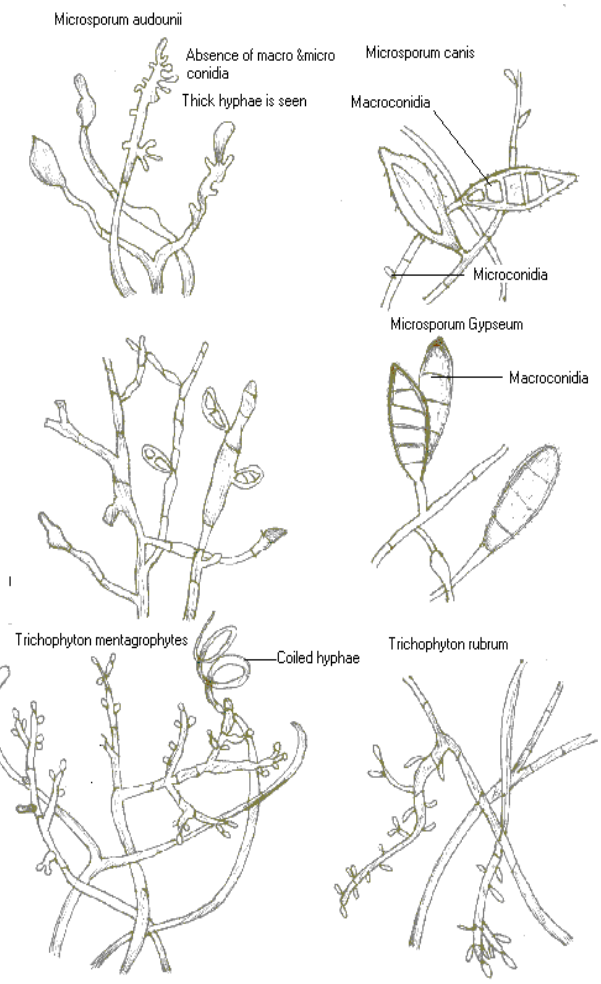
2) The non inflammatory type(which is difficult to eradicate) Hairy regions are particularly favourable for dermatophytic growth, since the hair is composed of nonliving keratin.

Dermatophytes affecting the hair of the scalp, eye brows and eye lashes is called Tinea capitis. It may be non inflammatory or markedly inflammatory with scarring. The terms Ectothrix and Endothrix refers to the location of the Arthroconidia that infect the hair. In Ectothrix (outside hair) invasion, the hyphae fragments into Arthroconidia that accumulate around the hair shaft or just beneath the cuticle, destroying it. The arthroconidia that are outside the hair shaft form a mosaic sheath, a pattern of arthroconidia resembling a tile mosaic. Hair invaded in ectothrix fashion typically becomes grayish, dull and discoloured, eventually the hair becomes brittle and break off. When many hairs have been lost in this fashion, irregular grayish areas are left on the scalp, resulting of gray patch Tinea capitis.

The Tinea capitis is not inflammatory, but scaling of the scalp is prominent feature of the infection. Gray patch Tinea capitis occurs primarily in childrens. The Endothrix (Inside) invasion, Arthroconidia form by fragmentation of the hypha within the hair shaft. The cuticle is not destroyed. Arthroconidia are not seen. The presence of the conidia weakens the hair so that it loses its lustur, becomes brittle and breaks off above the surface of the scalp. The conidia in the shafts of the hair appear as black dots. So it is called as Black dot Tinea capitis. The dermatophytid or ‘id’ reaction occurs in some peoples as an allergic response to a dermatophyte infection elsewhere in the body. The ‘id’ lesions donot contain the fungi causing the dermatophytosis but they are itchy and sometimes painful

10.4.1.5. Laboratory Identification

Specimen Collection



Skin should be **scraped** from the margin of the lesion onto folded black paper.

Hair should be **plucked**, not cut, from the edge of the lesion. Choose **hairs that fluoresce under a Wood's lamp** or, if none fluoresce, choose *broken or scaly ones.*

Nails clipings are obtained from the nail bed or from infected areas after the outer layers are discarded.

Direct Examination

A small volume of the specimen is selected for direct microscopic examination and investigated for the presence of fungal elements.

The specimen is mounted in a small amount of 10% or 20% Potassium Hydroxide or CALCOFLUOR white.

The KOH slides are gently heated and allowed to clear for 30 to 60 minutes before examining on a light or phase contrast microscope.

Calcofluor white slides are examined on a Fluorescent microscope.

or the production of pigment.

10.4.1.6. Treatment

Treatment is with topical ointments such as miconazole, tolnaftate or clotrimazole for 2 to 4 weeks. Griseofulvin and itraconazole are the only oral fungus agents currently approved.

Check your progress

10.4. What is dermatomycoses.

10.5. What is conidium

10.6. What is woods lamp test.

10.5. SUBCUTANEOUS MYCOSES

10.5.1. Sporotrichosis

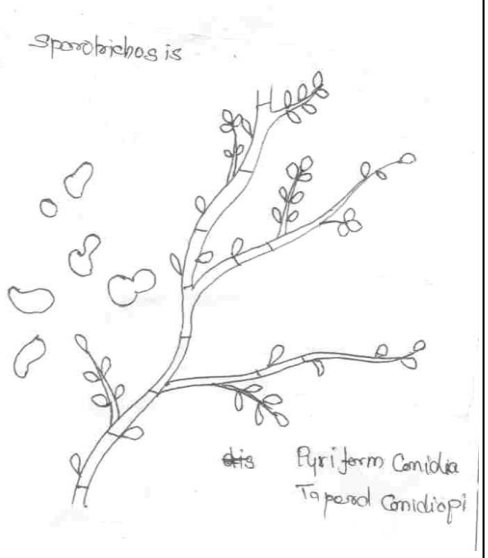
10.5.1.1. Introduction

It is one of the subcutaneous infections. It is a chronic granulomatous infection of human skin. This disease is world wide in

distribution. Etiology of Sporotrichosis was first described early periods of last century (1900s).

10.5.1. 2. Causative agent

It is caused by *Sporothrix schenckii*. Etiological agent is thermally dimorphic fungus. Because of the association of the organism on vegetation the disease is also called 'Rose Thorn Disease'. The fungus is Saprophytic in nature. Organism is found on plants thorns and timber. Infection is acquired through thorn pricks or other minor injuries.



10.5.1. 3. Incubation Period

It is highly variable, ranging from few days to months. Average is three weeks.

10.5.1. 4. Pathogenesis

Pathogen is temperature sensitive one. Temperature of skin surface and connective tissue are slightly lower than that of deep tissue. This will support the growth of etiological agent.

Mycelial fragments, conidia or any fraction of the *Sporothrix* is implanted into the deep layers of the skin through trauma.

Initially small hard painless nodule appear at the site of injury and it enlarges into a fluctuant mass that eventually breakdown and ulcerates.

The Subcutaneous nodule becomes discoloured and overlaying skin darkens to a reddish colour and eventually blackens.

Primary lesions enlarge and several nodules begin to develop along with lymphatics. Infection rarely extends beyond regional lymphatics. After few weeks the primary lesions will heals. The entire clinical disease is commonly called *Lymphocutaneous Sporotrichosis.* 75% of human infections are *Lymphocutaneous Sporotrichosis* Immunity of an individual reduce the severity of the infection.

Systemic spread is very low but dissemination may occur especially in debilitated patients. Following primary nodule, multiple subcutaneous nodule develop along the lymphatics channel and they become hard. It is called *Chronic Sporotrichosis.*

Fixed Sporotrichosis refers to the presence of only one lesion. It is a nonlymphatic nodule. It is less progressive, common in endemic areas. *Primary Pulmonary Sporotrichosis* may results from inhalation of the conidia.

10.5.1. 5. Epidemiology

Incidence is high among agricultural workers. It is an occupational risk disease. Highest prevalence of the infection was observed in Mexico. Animals are susceptible to this disease but spreading

NOTES

from animal to man is not observed. 75% of infected individuals are males.

10.5.1. 6. Laboratory Diagnosis

Specimen - Aspirated fluid, Pus, Biopsy tissue, Exudative material.

Microscopic examination - Specimens examined directly by KOH mount. Calcofluor white stain also used. In infected tissue, the fungus is seen as cigar shaped yeast cells without mycelium. *Asteroid bodies* are seen in lesions, composed of central fungus cells with eosinophilic materials radiating extensions. It is a antigen and antibody complex along with complement.

***Cultivation* -** Clinical specimen is inoculated into the inhibitory agar or antibiotics containing Sabourad dextrose agar and incubated at 25 –35°C. Colonies are observed within 1-3 days. Young colonies are blackish and shiny, becoming wringled and fuzzy with age. If the medium is incubated at 37°C yeast cells are seen.

Microscopic examination of colonies shows thin, branching mycelium, septate hyphae with small conidia and flower like arrangements are formed on delicate sterigmata.

Serology - Specific antibodies are not observed in the early stages of infection. Serological test is of little help in the diagnosis. Agglutinin to the yeast cell can be used to monitor the course of infection. Sporotrichin will elicit delayed skin reaction in sensitive person.

10.5.1. 7. Treatment

Oral solution of saturated Potassium Iodide is given. Dosage is increased daily at 0.5-1ml. Surface lesions are treated with 2% Potassium Iodide in 0.2% iodine. Other antifungal agents involved in the treatment purpose are Griseofulvin, Amphotericin B. Flucystosine, Dihydroxystilbamide, Ketaconazole

10.5.2. Chromoblastomycoses

10.5.2.1. Introduction

It is caused by dematiaceous fungi, which are imperfect fungi that produce varying amount of melanin like pigments. These pigments found in the conidia or hyphae or both

10.5.2.1.Causative agent

Phialophora veruucosa, Cladosporium carrionii, Fonsecae petrosoi, Fonsecae compacta and Rinocldiella aquaspersa

Melanin production is associated with the virulence of the etiological agents. It is caused by traumatic implantation of any one or several dematiaceous fungal species. The infection is chronic and characterized by the slow development of verrucous , cutaneous vegetation. The natural reservoir of these fungi is soil and plant debris. Dematiaceous fungi are similar in their pigmentation in their antigenic structure , morphology and physiological properties. The colonies are compact deep brown to black and develop a velvety often wrinkled surface. The agents of Chromoblastomycosis are identified by their mode of conidiation. In tissue, it produces spherical brown cells termed muriform or sclerotic bodies that divide by transverse septation.

Phialophora-conidia are produced from flask shaped philides with cupshaped collarettes.

Phialophora veruucosa **-** It produces branching chains of conidia by distal budding and also it produce elongated conidiophores with long branching chains of oval conidia

Rhinocladiella aquaspersa **-** It produce lateral or terminal conidia from a lengthening conidiogenous cell.

Fonsecae petrosoi **-** It is a polymorphous genus. It produce phialides similar to Cladosporium.

Fonsecae compacta **-** It produce spherical with a broad space connecting the conidia. Structures are smaller and compact.

10.5.2.3. Pathogenesis

Fungi are introduced in the skin by trauma , often of the exposed legs or feet . Over months to years the primary lesions become verrucous and wart like extension along the draining lymphatics. This is due to mononuclear cellular infiltrate. Cauliflower like nodules with crustin abscess eventually cover the area. Small ulceration also formed within short duration, these lesions become raised and appears scally and dull red or grayish in colour.

- Patients experienced minimal discomfort.
- Systemic invasion is extremely rare.

10.5.2.4. Lab Diagnosis

Specimen - Skin scrapings, 10% KOH mount is performed,Detection of sclerotic body is a main diagnostic tool.

10.5.2.5. Treatment

Surgical exision with wide margin is the therapy. Fluconazole, Itaconazole is also recommended.

10.5.3. Mycetoma

10.5.3.1. Introduction

Mycetoma is a chronic subcutaneous infection induced by traumatic inoculation with any of several saprophytic fungus. The disease is first observed in Madurai district of Tamilnadu. So this disease is also called as Madura foot and Maduramycosis. The clinical features defining Mycetoma are local swelling and interconnecting, often draining sinuses that contains granules, which are Microcolonies of the agent embedded in tissue material.

10.5.3.2. Causative agent

Madurella grisea, Phialophora jeanselinei, Leptosphaeria senegalensis, Cephalosporium sp., Acremonium sp., Petriellidium boydii and Pseudallescheria boydii.

10.5.3.3. Pathogenesis

Mycetoma develops after traumatic inoculation of any one of the agents of Mycetoma. Subcutaneous tissues of the foot, lowed

extremities, hands and exposed areas are most often involved in Mycetoma formation.
Pathology is characterized by supporation and abscess formation, granulomas and the formation of draining sinuses containing the granules.

10.5.3.4. Lab Diagnosis
Specimen : Pus, Exudates or Biopsy material
The granule colour, texture, size and the presence of hyaline or pigmented hyphae are helpful in determining etiology.
Culture - Specimen is inoculated into SDA with antibiotics
***Madurella mycetomatis* -** Colonies are smooth or folded, glabrous or powdery, leathery and range in colour from white to yellowish brown. Conidia are small, ovoid to globose. It also produce septate hyphae with many chlamydospores.
***Madurella grisea* -** Colonies are rapidly growing, gray or olive coloured. Gray aerial hyphae may be formed. Older colonies become reddish brown.the hyphae are brown walled septate. There of mycelia of too width and chains of arthroconidia.

10.5.3.5. Treatment
Management of Mycetoma is difficult. Amphotericin B is recommended. Katoconazole, Nystatin, Flucytosine, KI and Miconazole also used.

10.5.4. Lobomycosis

It is a chronic subcutaneous infection of humans and dolphin caused by a fungus called *loboa loboi.*It is first observed in 1931. Most patients are Adults and Male. Other names of the disease are lobo's disease or Keloid blastomycosis. Infection is restricted to Dermis. Lymph nodes are not involved. The infection is chronic and slow progressive. Lesions are not painful but ulcerative. The size of the lesions depends on the duration of the infection. The initial lesions are small hard subcutaneous nodules usually appearing on the extremities, face or ear, presumably as a result of traumatic inoculation. Inflammatory lesions show many foamy macrophages and multinucleated giant cells. Many of the phagocytes contain intact or fragmented cells. The lymphocytes and plasma cells appear on scattered clusters. Diagnosis is by direct microscopic examination of the skin scrapings, biopsy and wet preparation of exudative lesions. The fungus appears in tissue as large spherical or oval yeast that exhibit multiple budding. They are multinucleated and thick walled. Occasionally asteroid bodies are observed. Not successfully cultured in the laboratory. Acid schiff or Methenamine silver stain is used for staining. For the treatment, infected tissue must be carefully removed. Sulfa drugs are used for this purpose

10.5.5. Phaeohyphomycosis

It is a term used to apply for infections characterized by the presence of darkly pigmented septate hyphae in tissue. Both Systemic and Cutaneous mycosis have been described. The name Subcutaneous

Phaeohyphomycosis is because the causative agents are seen below the Dermis. Disease is caused by dematiaceous fungus like

Exphiala jeanselmei
Wangiella dermatitidis
Exphiala spinifera
Phialophora hoffmanii
Phialophora. repens
Phialophora . richardsiae
Tetraploa aristata
Bipolaris spicifera

All the fungus are exogenous mould normally exists in nature. During phaeohyphomycosis, cyst usually develops and may enlarge to several centimeters. Based on the pathogenisity, Phaeohyphomycosis is classified into four types.

Cutaneous Phaeohyphomycosis - Colonized on skin. May be seen over cracked, fissured areas of sole of foot. Ulceration is observed.

Subcutaneous Phaeohyphomycosis - Localized infection following deep inoculation of the fungus in to subcutaneous tissues. The lesions may occur on feet, legs, hands, arm etc. nodules are developed.

Systemic and Cerebral Phaeohyphomycosis - These are rare and are the most serious form of this disease. Cerebral Phaeohyphomycosis often present with brain abscess. The frontal lobes are mostly affected and encapsulated. Abscesses filled with brown hyphae are present in neurological biopsy.

Para nasal sinus Phaeohyphomycosis - It is an indolent disease found in sinus cavity and may spread to the adjacent areas.

Lab Diagnosis

Microscopy - *Abscesses are examined in KOH mount. These fungus are pigmented and dark brown because of melanin pigment. In tissue, the hyphae are large and often distorted and may be accompanied by yeast cells.*

Culture - *Clinical specimens are plated on SDA with Cycloheximide and are incubated at 25-37^{0}C. Most agents grow slowly and growth is visible only after one or two weeks.*

Exophiala jeanselmei and *Exophiala spinifera* produce black and slimy colonies with many yeast like cells. Hyphae are brown septate.

Phialophora hoffmanii, Phialophora. repens and Phialophora richardsiae produce Phialides with collarettes.

Wangiella dermatitidis - Young colonies are black, soft, moist shiny and yeast like

Treatment

Itraconozole and Amphotericin B are used for treatment

10.5.5. Rhinosporidiosis

Rhinosporidiosis is a chronic infection characterised by the development of polypoid masses of the nasal mucosa. It is a granulomatous disease of the mucocutaneous tissue of Man. It is caused by *Rhinosporidium seeberi*. This disease is first reported during 1990 from Argentina. Over 200 cases have been recognized. 90% of cases from India and srilanka. 905 of cases are Male. It produces large spherules in lesions and epithelial cells. It produces three types of disease in humans. They are Cutaneous Rhinosporidiosis, Nasal Rhinosporidiosis and Ocular Rhinosporidiosis

Life Cycle And Pathogenesis

The organism starts its life cycle in the tissue as rounded or oval spore with a cell membrane and clear cytoplasm. It undergoes some developmental process and is converted into mature spore. The organism develops into a sporangium containing endospores and having a thick outer chitinous wall. The spores escapes from the sporangium and then carried to the lymphatics. Lesions are most often found in the mucosa of the nose,nasopharynx and soft palate. Lesions are initially flat but develop into discoloured cauliflower type polypoid masses. In nasal area respiration may be blocked and is a profuse seropurulent discharge. During Ocular Rhinosporidiosis blood stained discharge may be noted. Large lesions may prevent occlusion of the eyelids causing exposure conjunctivitis.

Lab Diagnosis

Histologic tissue examination reveals epithelial hyperplasia and cellular infiltrate of neutrophils, lymphocytes , plasma cells and giant cells. Large thick walled sporangia also present. It is packed with thousands of endospores. The cellwall of spherule is multilayered. At maturation cellwall thin and endospores are liberated.

Treatment

Ethylstibamide is used for treatment

10.5.7. Phycomycosis

It is a chronic self limiting infection of the subcutaneous tissue caused by *Bacidiolobus haptosporus*. It was first observed in 1956. The colony is colourless or browinish thin flat glabrous. Fungus produce aerial mycelium. The hyphae are 8-20 μm wide and produce chlamydospores forcibly ejected spores and spherical smooth walled Zygospores. Highest incidence was observed among childrens between 5-9 years. 70-80% of infected individuals are Male. Infection begins on limb with a small firm movable nodule in the subcutaneous tissue. The nodule enlarges and oedema develops and may become massive involving an entire leg or shoulder. The skin become rough. Lesions are not painful. They persist for several months then resolve spontaneously. Direct microscopic examination of tissue reveals multiple granular giantcells and eosinophiles.

Broad hyaline branching hyphae with infrequent septa are surrounded by eosinophilic material. Culture on SDA media with out cycloheximide at

25^0C will yield colonies within 2-3 days. Potassium iodide is used for treatment.

Check your progress

10.7. What is SDA.

10.8. What is the use of KOH mount

10.9. What is itaconazole

10.10. Name the causative agent of Lobamycoses

10.6. LET US SUM UP

Pityriasis Versicolor is also called ***Tinea versicolor***. It is a chronic and less serious infection of the skin. This infection involves **stratum corneum** characterized by discoloration or depigmentation of skin. It is caused by *Malassezia furfur.* It is a superficial skin infection. It affects external appearance of an individual. It produces non-inflammatory macular lesions with fine scaling. Lesions are not usually itchy. Direct microscopic observation of the specimen with 10-20% KOH. Short unbranched hyphae and spherical cells are observed. Lesions also fluorescence under woods lamp test . Modified SDA supplemented with olive oil with antibiotics is used for cultivation. *M.furfur* is a lipophilic yeast. *Treatment* is by the application of *Selinum sulfide*

Tinea nigra is a superficial, chronic and asymptomatic infection of the Stratum Corneum caused by the dematiaceous yeast *Exophiala werneckii*. Lesions usually consist of a solitary, innocuous macuole with sharply defined margins. The brownish colour of the lesion is darkest at the advanced periphery, where most of the actively growing organisms are located. Specimens are mounted by 20% KOH or Calcofluor white stain. Observation reveals brown pigmented septate hyphae and budding yeast cells. Culture with Sabuourds Dextrose Agar with or without antibiotics should recover the organism. It responds well to topical keratolytic solutions of sulfur, salicylic acid or tincture of iodine.

Piedra are two types of Piedra. Black piedra and white piedra . Black Piedra is a Nodular Infection of the Hair shaft caused by *Piedraia hortae***.**. Color of the colony on SDA is greenish black or black. Hard nodules found along with the infected hair shaft. Nodules have a hard carbonaceous consistency**.**Hair is subjected to diagnosis. SDA medium is used for cultivation. Woods lamp test also performed. White Piedra is caused by *Trichosporan beigelii*. Culture is dimorphic one. Yeast cultures are white and have a pasty consistency. As the culture ages, colonies develop deep radiative farrows and take on a yellowish coloration with the creamy texture. Microscopic examination reveals septate hyphae that fragments rapidly to form arthroconidia. It is accomplished by the topical use of kerotolytic agents, preparation containing Selenium disulphide, Hyposulfite, Thiosulphite of salicylic acid. Miconazole nitrate is also used. Shaving or Cropping of infected hairs close to the scalp surface achieves effective therapy.

Dermatophytosis is fungal infections that involve the superficial areas of the body. The cutaneous infection caused by dermatophytes is called Tinea or Ringworm infection. On the basis of Clinical, Morphological and Microscopic characteristics, three different genera are

recognized as dermatophytes. They are Epidermophyton, Microsporum and Trichophyton. These fungi invade only dead corinified layers of the skin, hair and nails. Only a few organisms are necessary to induce an infection following trauma. Dermatophytes grow in filamentous form within the stratum corneum. The downward extension of this hyphae is restricted because certain nutrients, principally iron, are not available in the deeper tissues. Consequently, lateral invasions from the focus of inoculation occur, leading to the annular pattern seen on the skin surface. Treatment is with topical ointments such as miconazole, tolnaftate or clotrimazole for 2 to 4 weeks. Griseofulvin and itraconazole are the only oral fungus agents currently approved.

Sporotrichosis is caused by *Sporothrix schenckii* . *Incubation Period is* few days to months. Mycelial fragments, conidia or any fraction of the *Sporothrix* is implanted into the deep layers of the skin through trauma. Initially small hard painless nodule appear at the site of injury and it enlarges into a fluctuant mass that eventually breakdown and ulcerates. The Subcutaneous nodule becomes discoloured and overlaying skin darkens to a reddish colour and eventually blackens. Oral solution of saturated Potassium Iodide is givenOther antifungal agents involved in the treatment purpose are Griseofulvin, Amphotericin B. Flucystosine, Dihydroxystilbamide, Ketaconazole

Chromoblastomycoses is caused by dematiaceous fungi, *Phialophora veruucosa, Cladosporium carrionii, Fonsecae petrosoi, Fonsecae compacta and Rinocldiella aquaspersa.* Melanin production is associated with the virulence of the etiological agents. It is caused by traumatic implantation of any one or several dematiaceous fungal species. The infection is chronic and characterized by the slow development of verrucous , cutaneous vegetation. Fungi are introduced in the skin by trauma , often of the exposed legs or fee . Over months to years the primary lesions become verrucous and wart like extension along the draining lymphatics. Surgical exision with wide margin is the therapy. Fluconazole, Itaconazole is also recommended.

Mycetoma is a chronic subcutaneous infection induced by traumatic inoculation with any of several saprophytic fungus. Mycetoma is classified into three. Actinomycetoma is a Mycetoma caused by Actinomycetes. Mycetoma develops after traumatic inoculation of any one of the agents of Mycetoma. Subcutaneous tissues of the foot, lowed extremities, hands and exposed areas are most often involved in Mycetoma formation. Pathology is characterized by supporation and abscess formation, granulomas and the formation of draining sinuses containing the granules. The granule colour, texture, size and the presence of hyaline or pigmented hyphae are helpful in determining etiology. Management of Mycetoma is difficult. Amphotericin B is recommended. Katoconazole, Nystatin, Flucytosine, KI and Miconazole also used.

Lobomycosis is a chronic subcutaneous infection of humans and dolphin caused by a fungus called *loboa loboi.* The infection is chronic and slow progressive. Lesions are not painful but ulcerative. The initial lesions are small hard subcutaneous nodules usually appearing on the extremities, face or ear, presumably as a result of traumatic inoculation. Inflammatory lesions show many foamy macrophages and

multinucleated giant cells. Diagnosis is by direct microscopic examination of the skin scrapings, biopsy and wet preparation of exudative lesions. Acid schiff or Methenamine silver stain is used for staining. For the treatment, infected tissue must be carefully removed. Sulfa drugs are used for this purpose

Phaeohyphomycosis is a term used to apply for infections characterized by the presence of darkly pigmented septate hyphae in tissue.

Abscesses are examined in KOH mount. These fungus are pigmented and dark brown because of melanin pigment. In tissue, the hyphae are large and often distorted and may be accompanied by yeast cells.Clinical specimens are plated on SDA with Cycloheximide and are incubated at 25-37^{0}C. Itraconozole and Amphotericin B are used for treatment

Rhinosporidiosis is a chronic infection. It is a granulomatous disease of the mucocutaneous tissue of Man. It is caused by *Rhinosporidium seeberi.* The organism starts its life cycle in the tissue as rounded or oval spore with a cell membrane and clear cytoplasm. It undergoes some developmental process and is converted into mature spore. The organism develops into a sporangium containing endospores and having a thick outer chitinous wall. The spores escapes from the sporangium and then carried to the lymphatics. Histologic tissue examination reveals epithelial hyperplasia and cellular infiltrate of neutrophils, lymphocytes , plasma cells and giant cells. Ethylstibamide is used for treatment

10.7. UNIT END EXERCISES

Two Mark Questions

Mycoses
What are the two piedra that infects human
What is woods lamp test
Define KOH mount
What is LPCB
Mould
Yeast
Which fungus is called mycoses of implantation
Exophiala Wernekii
Miconazole
Dermatophytes
Calcoflour White
Sporotrichoses
Phycomycoses

Five Mark Questions

Give the general characters of fungus
Explain morpholical forms of fungus.
Describe tinea versicolar.
Explain Labamycoses
Describe Rhinosporidiasis
Give an account on diagnosis of fungal infection.
What are the microscopic methods available to study fungus from human sample.

Summarise treatment of fungal infections.

Ten Mark Questions

Write an essay on superficial mycoses.
Give a detailed note on dermatophytic fungi
What is Sporotrichsis? Explain.
Give a brief note on Mycetoma

10.8. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

10.1. By Producing Carbolic acid
10.2. Selenium sulfate is a drug for superficial mycoses.
10.3. dermatomycoses is also called ring worm infection
10.4. Dermatomycoses is a cutaneous mycotic infection caused by Micrsporum, Trichphyton And Epidermophyton
10.5. It is fruiting structure of fungus hels in identification of fungus.
10.6. Woods lamp test is used for identification of dermatophytes using UV lamp.
10.7. Saboured Dextrose agar used for fungal cultivation
10.8. KOH mount is used for assessing fungal infection in skin.
10.9. Itaconazole is a antifungal drug.
10.10. Loba lobai

10.9. SUGGESTED READINGS

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UNIT- XI MYCOSES

NOTES

Summary

11.1 INTRODUCTION

Systemic mycoses are invasive fungal infection. These infections result from inhalation of the spores of dimorphic fungi that have their mold forms in the soil. Opportunistic Systemic Mycoses. These are fungal infections of the body which occur almost exclusively in debilitated patients whose normal defence mechanisms are impaired. The organisms involved are cosmopolitan fungi which have a very low inherent virulence.

11.2 OBJECTIVES

Learning this chapter will be able to

Understand opportunistic and systemic mycotic infections of human.

11.3. SYSTEMATIC MYCOSIS

11.3.1. Coccidioidomycosis

11.3.1.1. Introduction

Coccidioidomycosis is an infection caused by the dimorphic fungus called *Coccidioids immitis*. The infection may be inapperant, benign, severe or even fatal. It is the endemic disease in the dry, arid regions of USA. Infection is acquired through inhalation of dust containing arthrospores of the fungus. Etiological agent is a dimorphic fungus that normally lives in soil. The organism was discovered in 1892 in tissue from fatal case and was named *Coccidioides* (coccidian like) the species name *immitis* means not mild. It is a disseminated disease. Smith discovered natural reservoir for *Coccidioids immitis*.

11.3.1.2. Causative agent

It grows in the media as mold. It produces a white, grey or brownish colour, powdery to cottony texture colonies. Microscopically colonies are observed as hyaline branching septate hyphae and as the culture ages, characteristic arthroconidia are produced. In older cultures, hyphae fragments releases unicellular barrel shaped arthroconidia. Arthroconidia are highly resistant to desiccation, temperature extremes and deprivation of nutrients and may remain viable for years. In the infected host, *Coccidioids immitis* exist as spherules, a spherical thick

walled structure 15-18 μm in diameter, that are filled with a few to several hundred endospores. As the spherule enlarges, the nuclei undergoes mitosis, the cytoplasm condenses around the nuclei and cellwall forms around each developing endospores. At maturation,the spherules ruptures to release its endospores. These endospores enlarge and to form mature spherule. It is observed in tissues and may appear in sputum of patients with Coccidioidal cavities in lungs.

11.3.1.3. Antigenic Structure

Coccidioidin is a crude antigen extracted from the filtrate of liquid mycelial culture of *Coccidioids immitis*. **Spherulin**, produced from a filtrate of broth culture of spherules. Both antigens are positive to delayed skin reactions. HS, F and HL are some of the exoantigen of *Coccidioids immitis* .

11.3.1.4. Pathogenesis

Inhalation of arthroconidia leads to a primary infection. Respiratory tract is a major site for the infection. 60% of the infections are asymptomatic. The cellwall of the infectious particle has several layers. Outer layer represents the original cellwall, middle layer is called thin fibrous rodlets and inner thick wall. Outer layer and rodlet layer contains mannon, protein and lipid and are readily solubilized. The inner layer contain chitin, 3 amino methyl mannose. When arthroconidia develop into spherules, it releases an antigen. Arthroconidia and endospores are readily engulfed by alveolar macrophages and kill *Coccidioids immitis.* Activation of macrophages enhances killing activity of *Coccidioids immitis.* Primary Coccidiodomycosis has a incubation period of 10-16 days. Upto 20% of the patients with Pulmonary Coccidioidomycosis manifests allergic reactions, usually Erythema nodosum.

Some patients may develop a chronic but progressive pulmonary disease with multiplying or enlarged cavities or nodules. In severe Coccidioidomycosis, patients having elevated antibody titres, circulating immune complexes and depressed cellular immunity. Recovery of infection often leads to restoration of immune functions. The impaired immune functions are due to the documented increase in the population of suppressor cells, blocking factors, immune complexes and impaired lymphocyte circulation. Immune complexes may contribute to the immunopathology by two mechanisms. Deposition of the complexes may leads to local inflammatory responses. Immunosuppresssions may results from the binding of complexes to cells bearing Fc receptors.

Chroic Coccidioidomycosis develops from initial lesions that appear on the face or neck. Osteomyelitis may also develop.

11.3.1.5. Symptoms

Fever, Chest pain, Cough, Weight loss, Extra pulmonary infection involves the meninges, skin or bone.

11.3.1.6. Lab Diagnosis

Specimen – Sputum, Exudates from cutaneous lesion, Spinal fluid, Urine and Tissue biopsy.

Microscopic examination - Clinical exudates should be examined directly in 10% or 20% KOH or Calcofluor white stains. Tissue specimens are stained with hematoxylin and eosin. Microscopic examination shows spherules and endospores.

Culture - Clinical specimens are inoculated into the Inhibitory Mold Agar or Sabourauds Agar with antibiotics like Cycloheximide, Chloramphenicol and Gentamycin. Media is incubated at room temperature or 37°C. Colonies of *Coccidioids immitis* may develop within 1 to 2 weeks and are examined microscopically for the production of characteristic Arthroconidia. Spherule formation is observed by the incubation of complex medium at 40^0C with 20% $CO_{2.}$

Immunodiffusion test demonstrates the presence of specific antigen.

The CF test for antibodies to Coccidioidin is a powerful diagnostic and prognostic tool.

Skin testing - The Coccidioidin skin test reaches its maximum duration between 12-48 hours after cutaneous injection of 0.1ml of a standardized dilution.

11.3.1.7. Epidemiology

Organisms are most prevalently observed in semiarid climate, alkaline soil and characteristic indigenous desert plants and rodents. Coccidioidomycosis is considered as a occupational hazard for construction workers, archeology students.

11.3.1.8. Treatment

Symptomatic primary infections are self-limited and require only supportive treatment. Patients with severe disease require treatment with Amphotericin B, which is administered intravenously.

11.3.2. Histoplasmosis

11.3.2.1. Introduction

Histoplasmosis is the most prevalent pulmonary mycosis of humans and animals. It is caused by the dimorphic soil saprophytic fungi

11.3.2.2. Causative agent

Histoplasma capsulatum. It is distributed throughout the world. Infection is initiated by inhalation of fungus conidia. *Dr.Samuel Darling* discovered Histoplasmosis and also gives clear-cut description of the disease. This disease is also called **Darlings disease**.

Two colonial forms are produced during cultivation. They are type A or albinotype and B or brown type. Both phenotypes produce identical yeast and tissue forms. *Histoplasma capsulatum* is a thermally dimorphic fungus. At temperature below 35°C, it grows as a mold, often white or brown color and at 35°C. It grows as yeast with small heaped and pasty colonies. It grows very slowly. Under optimal condition the mold colony develops after 1 or 2 weeks.

Both Microconidia and Macroconidia are produced at temperatures below 37°C. The hyaline, septate hyphae produce Microconidia(2-5μm) and large thick walled Macroconidia with peripheral projections of cell wall material (8-16μm)

Histoplasma capsulatum is a facultative intracellular parasite. In tissue, yeasts are seen within macrophages. Histoplasmin is a antigen extracted from the *Histoplasma capsulatum.*

11.3.2.3. Pathogenesis

Etiological agent *Histoplasma capsulatum* is entered into the lungs through inhalation. Conidia develop into yeasts after settling of *Histoplasma capsulatum* mycelium in to the alveoli. Yeast cells are engulfed by alveolar macrophages. Within macrophage, yeast cells are able to multiply and are disseminated to reticuloendothelial tissues such as the liver, spleen, bone marrow and lymph nodes through blood stream.

Tissue reaction may involve an early infiltration of neutrophils and lymphocytes, which will leads to granulomatous inflammatory response and also produce epitheloid cell tubercle.

Acute Pulmonary Histoplasmosis - Symptoms ranging from a mild flu like illness that clears spontaneously to a moderate or severe disease. Incubation period varies from one to several weeks. Symptoms are fever cough, chest pain,dyspnea , hoarsness night sweats and weight loss

Chronic Pulmonary Histoplasmosis - It is most often seen in Males. It is considered to be an opportunistic complication of lung disease. Symptoms are low grade fever, a productive cough, progressive weekness and fatigue.

Disseminated Histoplasmosis - Dissemination may be completely benign and inapparent except for the calcified lesions, usually in organs of reticuloendothelial tissues. It may acute and progressive.

11.3.2.4. Symptoms

Symptoms are splenomegaly and hepatomegaly, weight loss, anemia and leucopenia. Granulomatous lesions and macrophages packed with yeast cells can be observed throughout reticuloendothelial systems.
Acute progressive Histoplasmosis is often fulminant and rapidly fatal. *Presumed Ocular Histoplasmosis Syndrome (POHS) is also observed in some cases.*

11.3.2.5. Lab Diagnosis

Specimen - Blood (buffy coat), Bone marrow, Sputum, Scrapings from the superficial lesions and Pus from sinus tract

Microscopic examination -Smears of infected specimens are fixed with methanol and stained with Wright or Giemsa stain will reveal characteristically ellipsoidal yeast cells inside of macrophages.

Culture - Purulent portion of the specimen should be selected for culturing. In endemic areas, specimens should be inoculated on at least 4 media. They are Sabourauds agar without antibiotics incubated at 37°C;Sabourauds agar with antibiotics incubated at 25- 30°C; Brain heart infusion agar with 5% sheep blood without antibiotics incubated at 37°C and Brain heart infusion agar with 5% sheep blood with antibiotics Cycloheximide incubated at 25- 30°C. pH of the media should be neutral, incubated atleast for 4 weeks because etiological agent grows very slowly.

H . capsulatum is identified by characteristic macroconidia at 25-30°C and observation of yeast cells at 37°C.

Skin test - Histoplasmin skin test is a valuable tool in epidemiology. Within two weeks after infection, most persons become skin test positive.

Serology - Two tests are widely used. They are complement fixation test and immunodiffusion test.

11.3.2.6. Treatment

Amphotericin B is a drug of choice. A total of 1.5g is recommended.

11.3.3. Blastomycosis

11.3.3.1. Introduction

This is a chronic infection characterized by granulomatous and suppurative lesions initiated by inhalation of a thermally dimorphic fungus, *Blastomyces dermatitidis*. This disease is also called north American blastomycosis because initial cases are confirmed to the united states. Blastomycosis is first described in its cutaneous form in the 1980s by Gilchrist . Hence Blastomycosis is also known as Gilchrists or Chicago disease. Blastomycosis is primarily a pulmonary infection characterized by spread to the skin and other parts of the body. Soil is considered to be the source of infection. Organism is acquired by inhalation.

11.3.3.2. Causative agent

Blastomyces dermatitidis is a dimorphic fungus. On sabourauds glucose agar at 25°C, the organism grows as a mould, producing a colony of uniform hyaline, septate hyphae and conidia. Colony development requires at least 2 weeks. Many strains produce a cottony mycelium that becomes tan to brown with age. On enriched media at 37°C , it grows as a yeast with folded pasty and moist colonies.

Microscopically the mycelial form produces abundant conidia from the aerial hyphae and lateral conidiophores. The conidia are spherical, ovoid or pyriform in shape and are 3-5μm in diameter.

Extracts of culture filtrates of *Blastomyces dermatitidis contain blastomycin.*

11.3.3.3. Pathogenesis

Blastomycosis are acquired by inhalation of exogenous, infectious particles. Initial site of infection is lungs. In the alveoli *Blastomyces dermatitidis* induces an inflammatory response characterized by the infilteration of both macrophages and neutrophiles and subsequent formation of granulomas. Both conidia and yeast cells are susceptible to the oxidative killing of mechanisms of neutrophiles and fungicidal activity of macrophages. Neutrophiles and CMI cooperate to produce effective resistance to blastomycosis Two classic forms of Blastomycosis are recognized-pulmonary Blastomycosis and chronic Cutaneous Blastomycosis.

Pulmonary Blastomycosis - It may be asymptomatic or may occur as acute or subacute pneumonia. It may also persist locally or spread to other organs.

Chronic Cutaneous Blastomycosis -The initial skin lesions appear as one or more subcutaneous nodules that eventually ulcerate. Lesions are most common on exposed skin surfaces such as face , hands and lower legs. If untreated , elevated granulomatous lesions with advancing borders will develop. The yeast cells can be found in microabscessess near the dermis

Disseminated Blastomycosis is most commonly involved in extra pulmonary sites. This infection may be chronic. From the lungs yeast spreads through blood stream. Primary cutaneous Blastomycosis is initiated by traumatic autoinoculation or contamination of open wound with the infectious material.

11.3.3.4. Symptoms

Symptoms are fever, malaise, night sweatsand cough. Pulmonary lesions heals by fibrosis and resorption.

11.3.3.5. Lab Diagnosis

Specimen - Sputum , Pus, Exudates, Urine

Microscopic examination - In calcofluor or KOH preparations of pus and sputum, a diagnosis can be made by detection of yeast cells. The yeasts are large and typically have thick cell wall. In tissue stained with hematoxylin and eosin, the yeast cytoplasm stains darkly and the cellwall appear colourless. The cells may be multinucleated.

Culture - Specimens are cultured on inhibitory mold agar or Sabourauds agar and sheep blood enriched media.

Skin test - Delayed type hypersensitivity has been detected.

Serology - The most useful serological procedure is an ID test for specific precipitins. An enzyme immunoassay for antibodies to antigen A is recently evaluated.

11.3.3.6. Treatment

Amphotericin B is effective against blastomycosis

11.3.4. Paracoccidiodiomycosis

11.3.4.1. Introduction

Primary site of the infection is lungs and then may spread to other parts and produce ulcerative granulomas in the mucosal surfaces of the nose, mouth and gastro intestinal tract. Internal organs may also become infected.

11.3.4.2. Causative agent

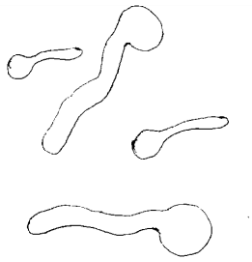
Paracoccidiomycosis or South American blastomycosis is a systemic mycotic infection caused by dimorphic fungus *Paracoccidioides braciliensis.* It is a chronic granulomatous infection. On SDA at 25-30°C, *Paracoccidioides braciliensis* grow very slowly, reaching a diameter of 1-2 cm after 2-3 weeks of incubation. Various conidia are produced by *Paracoccidioides braciliensis* including chlamydospores, arthroconidia and singly borne conidia. By growth on rich medium at 35-37°C, the yeast form can be induced. Yeasts are larger and have thinner walls than the yeast of *Blastomyces dermatitidis*. The

buds are attached by a narrow connection.multiple budding yeasts also formed. Yeast cells of *Paracoccidioides braciliensis* die after approximately 2-3 weeks in broth cultures. This is due to accumulation of toxic phenolic compounds.

11.3.4.3. Pathogenesis

*Paracoccidioides braciliensis i*s inhaled and initial lesions are observed in lungs. After inhalation, because of the body temperature of 37°C, mycelial form of the etiological agent is converted in to yeast form. Yeast is considered to be an active pathogenic agent and involved in dissemination. Yeast cellwall polysaccharides such as alpha glucan is associated with virulence. After a period of dormancy, which may lasts for decades, the pulmonary granuloma may become active, leading to chronic progressive pulmonary disease. Organism spread from lungs to other organs like skin, mucosubcutaneous tissue, lymphnodes, spleen, liver and other sites.

Many patients' presents with painful sores involving oral mucosa. The disease is common in male than females. Because a protein of *Paracoccidioides braciliensis* binds to the oestrogen but not testosteron or other hormones. Binding prevents conversion of mycelial form to yeast form at 37°C. It will explain the resistance of female against paracoccidiodomycosis.



11.3.4.4. Symptoms

Lymphadenopathy, hepatosplenomegaly.
fever, weight lossand malaise; present in most patients.

11.3.4.5. Lab Diagnosis

Sample - Sputum, Tissue, Scrapings
Microscopy - Specimens are observed by KOH mount or Calcoflour method. Wet preparations are examined for yeasts
Culture **-**Culture is performed by SDA and incubated at 25 and 37^0C
Serology **-** Both ID and CF tests are done

11.3.4.6. Treatment

Ketoconazole is the drug of choice. Amphotericin B is also effective. Sulfa drugs such as sulfa methoxy pyridazine.

11.4. OPPORTUNISTIC SYSTEMIC INFECTION

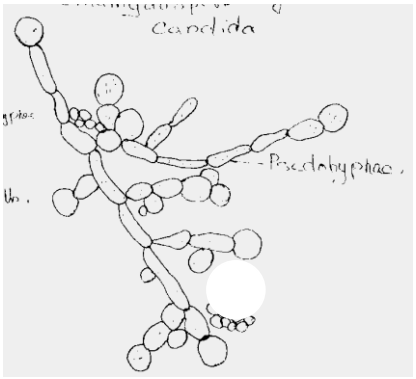
11.4.1. Candidiasis

11.4.1.1. Introduction

The members of the genus Candida cause Candidiasis. These organisms are the members of the normal flora of the skin, mucous membranes and gastrointestinal tract. It occurs worldwide and is the most common systemic mycosis. Of more than 100 species of candida, *Candida albicans c*ause most infection followed by *Candida tropicalis.*

11.4.1.2. Causative agent

Candida albicans is capable of producing yeast cells with pseudohyphae and true hyphae. In serum at 37^0C, *Candida albicans* produce true hyphae, that technique is called germ tube technique.



On most media they produce raised, cream coloured opaque colonies within 24-48 hours.

It produces ellipsoidal or spheical budding yeasts about 3-6μm in size.

Speciation -Two serotypes of *Candida albicans*, designated as A and B

Determinants of pathogenecity

Hyphal production and resistance to phagocytic killing is associated with virulence. High doses of extracts of *Candida albicans* exhibit endotoxin activity. Germ tubes are more adhesive than yeast cells. Most strains produce protease enzyme that cleaves immunoglobulins.

11.4.1.3. Pathogenesis

Cutaneous mucosal Candidiasis - Superficial Candidiasis is established by an increase in the total census of Candida and damage to the skin or epithelium that permits local invasion by the yeast and pseudohyphae.

Oral thrust can occur on tongue , lips, gums or palatic . It is a patchy to confluent whitish pseudomembranous lesion composed of epithelial cells yeast and pseudohyphae.

Yeast invasion of the vaginal mucosa leads to vulvovaginitis characterized by irritation, pruritus and vaginal discharge.

Candidial invasion of the nails and around the nail plate causes only chomycosis, a painful erythematous swelling of the nail fold resembling a pyogenic parongchia, which may eventually destroy the nail

Systemic Candidiasis- It occurs when Candida enters the blood stream and the phagocytic host defences are inadequate to control the growth and dissemination of the yeasts. Host defences against Candidiasis are both specific or non specific, cellular and humoral serum components such as opsonins, complement, transferin may inhibit the survival of Candida.

Numerous systemic manifestations of Candida may follow introduction of Candida in to the blood stream. Esopharyngitis, intestinitis infant diarrhoea, bronchopulmonary candidiasis, pylonephritis, cystitis, endocarditis, myocarditis, endoopthalmitis, meningitis, orthritis, osteomyeletis, peritonitis, macronodular skin lesions.

Candidemia may results from contamination of indwelling catheters, surgical procedures,trauma to the skin etc. Candidiasis of the mucous

membrane often referred to as thrush. Vaginal thrush occurs more often in pregnant women.

11.4.1.4. Lab Diagnosis

Specimen – Scrapings, Blood, Spinal fluid, Tissue biopsies, urine and Exudates.

Microscopy - Fluid specimens are examined in Gram stained smears for pseudohyphae or true hyphae along with budding yeast cells. Skin or nail scrapings are examined in a drop of 10% KOH and calcofluor white for hyphal forms.

Culture - Specimens are inoculated on SDA agar and incubated at 37^0 C for 24 –48 hours. Creamy colonies are observed if the specimen contains candida. Cvolies are observed for the presence of pseudohyphae.

11.4.1.5. Treatment

Cutaneous candidiasis treated with topical antimycotic substances like ketoconazole, nystatin, miconazole. For the treatment of systemic candidiasis amphotericin B , flucytosine are recommended.

11.4.2. Cryptococcosis

11.4.2.1 Introduction

Cryptococcosis is usually associated with immuno suppression. Disease is world wide in distribution. It is caused by encapsulated yeast . The natural reservoir for *Cryptococcus neoformans* is the soil and the avain feces and infection follows air borne exposure and inhalation of the yeast . It is ubiquitous in nature, the incidence of cryptococcosis is relatively low.

11.4.2.1 Causative agent

Visible colonies of *Cryptococcus neoformans* develop on routine laboratory media within 36-72 hours. They are white to cream coloured opaque and may be several millimeters in diameter. Colonies are typically mucoid in appearance and the amount of capsule produced can be judged by the degree of colony wetness.

Most clinical isolates are spherical budding encapsulated yeast cells in both tissue and cultures. Rarely short hyphal forms also observed. The hallmark of *Cryptococcus neoformans* is its capsule, which may be twice the width of the cell. Elevated glucose CO_2 or temperatures enhances capsule formation.

During infection or immunization with whole yeast cells antibodies are formed to the capsule. Four types of serotypes of are designated based on the capsulated antigens. They are serotypes A,B,C and D. A and B are designated as one variety, C and D designated as another one variety. *Cryptococcus neoformans* var neoformans represents serotype A and B. *Cryptococcus neoformans* var gatti corresponds with serotypes C and D. Serotypes are correlated with prevalence of the infection. *Cryptococcus neoformans* var neoformans is globally distributed and *Cryptococcus neoformans* var gatti is associated with eucalyptus trees of trophical countries.

Special feature of the pathogenic *Cryptococcus neoformans* is growing best at 37°C and inhibited at 41°C. All Cryptococcus species are nonfermentative, hydrolyse starch, assimilate inositol and produce urease

Cryptococcosis is a sporadic infection with a world wide in distribution. *Cryptococcus neoformans* is world wide in distribution in the soil and in Avian fecal material, which apparently provide a reservoir of organisms. Cryptococcosis occurs equally in both sexes.

11.4.2.3 Pathogenesis

The high prevalence of *Cryptococcus neoformans* in nature and relatively low frequency of disease suggests that many persons are probably exposed without any symptoms. Cryptococcosis is initiated in the lungs after inhalation of yeast cells of *Cryptococcus neoformans*. Based on symptoms there are two types of Cryptococcosis. They are Pulmonary Cryptococcosis and Disseminated Cryptococcosis.

***Pulmonary Cryptococcosis* -** Primary infection may be symptomatic or may an influenza like respiratory infection. It is rarely fulminant and hilar lymphadenopathy , calcification and cavitation are seldem observed. Symptoms are cough, sputum production , weight loss or fever.

***Disseminated Cryptococcosis* -** *Cryptococcus neoformans* is neurotrophic and disseminates to the central nervous system. Meningitis may be acute or chronic. Symptoms are fever, head ache, stiff neck and disorientation are accompanied by spinal fluid that typically is clear increased opening pressure, presence of cells.

11.4.2.4 Laboratory Diagnosis

***Specimens*-** Spinal fliuid, Aspirate, Skin lesions, Sputum, Tissue

Microscopy - Specimens should be examined directly in an Indian ink preparation for the presence of yeast cells with capsule. Encapsulated yeasts in tissue sections appear to the surrounded by large empty spaces because of the poor staining of the capsular polysaccaride.

Culture - Culture is performed by SDA and incubated at 37^0C

11.4.2.5. Treatment

Cryptococcosis is treated with both Amphotericin B and Flucytosine.

11.4.3. Aspergillosis

11.4.3.1. Introduction

Aspergillosis refers to a spectrum of diseases that may be caused by a number of Aspergillus species. Aspergillus species are ubiquitous in nature and are saprophytes. It occurs as worldwide. Some 150 different species and subspecies of Aspergillus have been recognized. They can be isolated from vegetation especially nuts and grains. *A.fumigatus* is the most pathogenic species for humans, although many species like *A.flavus* and *A.terrus* known to produce infection.

11.4.3.1. Causative agent

Aspergillus species grow very rapidly, producing aerial mycelium becomes powdery and pigmented conidia. Based on the color of the colony and morphology of conidia various species of Aspergillus are identified.

Microscopic Appearance

Aspergillus is characterized by conidiophores, which expand into large vesicles at the end and are covered with phialides that produce long chains of conidia. Phialides may arise directly from the vesicle.

11.4.3.3.Pathogenesis

Most cases of Aspergillosis develop in individual who have structural abnormalities with in the lungs or who have severely impaired resistance to infections

Inhalation of fungal mycelia leads to aspergillosis. Based on the type of infection aspergillosis is clinically classified into three types

- Allergic broncho pulmonary aspergillosis
- Aspergilloma and extra pulmonary colonization
- Invasive aspergillosis

In the lungs, alveolar macrophages are able to engulf and destroy the Conidia. Conidia swell and germinate to produce hyphae that have a tendency to invade preexisting cavities or blood vessel

Allergic Form -In some atrophic individuals, development of IgE antibodies to the surface antigens of Aspergillus conidia elicits an immediate asthmatic reaction. The conidia germinate and hyphae colonise the bronchial tree without invading the lung parenchyma. This phenomenon is a characteristic of allergic bronchopulmonary Aspergillosis. Symptoms are asthma, eosinophill accumulation, type I & II hypersensitivity reaction.

Aspergilloma - After the inhalation of conidia, it enters in the existing cavity, germinate and produce abundant hyphae in the abnormal pulmonary space. Patients with tuberculosis and sarcoidosis are at risk. Some patient develop cough, dyspnea, weight loss , fatigue and hemoptysis. Cases of Aspergilloma rarely become invasive. Many patients with Aspergilloma are asymptomatic.

Invasive Aspergilosis - Following inhalation and germination of the conidia, invasive disease develop as an acute pneumonic process with or without dissemination. Symptoms include fever, cough, dyspnea. Hyphae invade the lumen and walls of blood vessel causing thrombosis and necrosis.

11.4.3.4. .Lab Diagnosis

Specimen **-**Sputum, Lung biopsy

Microscopy - On direct microscopic examination of sputum with 10%KOH or Calcoflour white, the hyphae of Aspergilli are observed as hyaline, septate uniform width.

Culture - Aspergillus grows within few days on media at room temperature. Cycloheximide containing media should not be used.

11.4.3.5. Treatment

- Aspergilloma are treated with Amphotericin B
- Allergic forms are treated with Corticosteroids
- Itraconazole and Flucytosine have also been used

14.5. MYCOTOXICOSIS.

14.5.1. Introduction

Mycotoxicoses are acute and chronic toxic diseases caused by mycotoxins. Mycotoxins are produced by fungus. A mycotoxin is a toxic secondary metabolite produced by fungus. It causes morbidity and mortality in both humans and other animals. In general mycotoxin are produced in stored grains and crops. the disease associated with mycotoxin are called mycotoxicosis

14.5.2. Examples of mycotoxins

Aflatoxin, citrinin, fumonisins, ochratoxin A, Patulin, trichothecenes, ergotamine.

14.5.3. Chemistry and Biology of mycotoxins

Aflatoxins are produced by Aspergillus such as A. flavus, A. parasiticus. Four types of Aflotoxins are produced by Aspergillus. They are Aflotoxin B1, B2, G1and G2. Among these B1 is more toxic in nature. Aflatoxin B1is a potent carcinogen and has been directly correlated to adverse health effects such as liver cancer.

Ochratoxin is a mycotoxin produced by Penicillium and Aspergillus ochraceus. There are three types of Ochratoxin. They are ochratoxin A, B and C. The three forms differ from one another. Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form. *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including beverages such as beer and wine. Aspergillus carbonarius is the main species found on vine fruit, which releases its toxin during the juice making process. OTA has been labeled as a carcinogen and a nephrotoxinand has been linked to tumors in the human urinary tract.

Citrinin is a toxin that was first isolated from Penicillium citrinum. Citrinin is associated with yellowed rice disease. It is a nephrotoxin. This is associated with many human foods (wheat, rice, corn, barley, oats, ryeand food colored with Monascus pigment).

Ergot Alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of Claviceps, which are common pathogens of various grass species. The ingestion of ergot sclerotia from infected cereals, commonly in the form of bread produced from contaminated flour, causes ergotism. Ergotism is also called St. Anthony's Fire. It affects central nervous system.

Patulin is a toxin produced by the Penicillium expansum. Rotting of fruit and vegetables produce Patulin. Patulin is not a carcinogen.

Fumonisins are the mycotoxin produced by Fusarium sp. it affect the nervous systems of horses and may cause cancer in rodents. The trichothecenes are most strongly associated with chronic and fatal toxic effects in animals and humans. Zeralenone is another one mycotoxin. Beauvercin, enniatins, butenolide, equisetinand fusarins are the mycotoxins of Fusarium

Health effects

Health effects found in animals and humans include death, identifiable diseases or health problems, weakened immune and as allergens or irritants.

Mechanism

Mycotoxins entered human system via via ingestion, skin contact, inhalationand entering the blood stream and lymphatic system. They inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lungand increase sensitivity to bacterial endotoxin.

Check Your Progress

11.1. What is dimorphic fungus
11.2. What is sperulin
11.3. Name drug that is used for systemic mycoses.
11.4. What is candidiasis
11.5. What is Aspergilloma.
11.6. What is Systemic Mycoses
11.7. What is Darlings disease
11.8. What is Aflatoxin
11.9. What is Mycotoxicosis
11.10. What is neurotoxin

11.6. LET US SUM UP

Coccidioidomycosis is an infection caused by the dimorphic fungus called *Coccidioids immitis*. It grows in the media as mold. It produces a white, grey or brownish colour, powdery to cottony texture colonies. Microscopically colonies are observed as hyaline branching septate hyphae and as the culture ages, characteristic arthroconidia are produced **Coccidioidin** is a crude antigen extracted from the filtrate of liquid mycelial culture of *Coccidioids immitis*. Clinical exudates should be examined directly in 10% or 20% KOH or Calcofluor white stains. Tissue specimens are stained with hematoxylin and eosin. Microscopic examination shows spherules and endospores Patients with severe disease require treatment with Amphotericin B, which is administered intravenously.

Histoplasmosis is the most prevalent pulmonary mycosis of humans and animals. It is caused by the dimorphic soil saprophytic fungi *Histoplasma capsulatum.* This disease is also called Darlings disease.

Etiological agent *Histoplasma capsulatum* is entered into the lungs through inhalation. Conidia develop into yeasts after settling of *Histoplasma capsulatum* mycelium in to the alveoli. Yeast cells are engulfed by alveolar macrophages. Within macrophage, yeast cells are able to multiply and are disseminated to reticuloendothelial tissues such as the liver, spleen, bone marrow and lymph nodes through blood stream.

H . capsulatum is identified by characteristic macroconidia at 25-30°C and observation of yeast cells at 37°C. Histoplasmin skin test is a valuable tool in epidemiology. Within two weeks after infection, most persons become skin test positive. Amphotericin B is a drug of choice.

Blasto Mycosis is a chronic infection characterized by granulomatous and suppurative lesions initiated by inhalation of a thermally dimorphic fungus, *Blastomyces dermatitidis*. Blastomycosis is also known as Gilchrists or Chicago disease. Blastomycosis are acquired by inhalation of exogenous, infectious particles. Initial site of infection is lungs. In the alveoli *Blastomyces dermatitidis* induces an inflammatory response characterized by the infilteration of both macrophages and neutrophiles and subsequent formation of granulomas. The most useful serological procedure is an ID test for specific precipitins. An enzyme immunoassay for antibodies to antigen A is recently evaluated. Amphotericin B is effective against blastomycosis

Paracoccidiomycosis is the lungs ainfection. On SDA at 25-30°C, *Paracoccidioides braciliensis* grow very slowly, reaching a diameter of 1-2 cm after 2-3 weeks of incubation. *Paracoccidioides braciliensis i*s inhaled and initial lesions are observed in lungs. After inhalation, because of the body temperature of 37°C, mycelial form of the etiological agent is converted in to yeast form. Culture is performed by SDA and incubated at 25 and 37^0C. Ketoconazole is the drug of choice. Amphotericin B is also effective. Sulfa drugs such as sulfa methoxy pyridazine.

Candidiasis is the infection caused by *Candida albicans.* It is capable of producing yeast cells with pseudohyphae and true hyphae. On most media they produce raised, cream coloured opaque colonies within 24-48 hours. It produces ellipsoidal or spheical budding yeasts about 3-6μm in size. Specimens are inoculated on SDA agar and incubated at 37^0 C for 24 –48 hours. Creamy colonies are observed if the specimen contains candida. Cvolies are observed for the presence of pseudohyphae.Cutaneous candidiasis treated with topical antimycotic substances like ketoconazole, nystatin, miconazole. For the treatment of systemic candidiasis amphotericin B , flucytosine are recommended.

Cryptococcosis is usually associated with immuno suppression. Disease is world wide in distribution. It is caused by encapsulated yeast . The natural reservoir for *Cryptococcus neoformans*. Visible colonies of *Cryptococcus neoformans* develop on routine laboratory media within 36-72 hours. They are white to cream coloured opaque and may be several millimeters in diameter. Colonies are typically mucoid in appearance and the amount of capsule produced can be judged by the degree of colony wetness. Special feature of the pathogenic *Cryptococcus neoformans* is growing best at 37°C and inhibited at 41°C.. Based on symptoms there are two types of Cryptococcosis. They are

Pulmonary Cryptococcosis and Disseminated Cryptococcosis. Cryptococcosis is treated with both Amphotericin B and Flucytosine.

Aspergillosis refers to a spectrum of diseases that may be caused by a number of Aspergillus species. Aspergillus species grow very rapidly, producing aerial mycelium becomes powdery and pigmented conidia. Based on the color of the colony and morphology of conidia various species of Aspergillus are identified. Aspergillus is characterized by conidiophores, which expand into large vesicles at the end and are covered with phialides that produce long chains of conidia. Phialides may arise directly from the vesicle. Most cases of Aspergillosis develop in individual who have structural abnormalities with in the lungs or who have severely impaired resistance to infections Inhalation of fungal mycelia leads to aspergillosis. Based on the type of infection aspergillosis is clinically classified into three types. They are Allergic broncho pulmonary aspergillosis, Aspergilloma and extra pulmonary colonization and Invasive aspergillosis. On direct microscopic examination of sputum with 10%KOH or Calcoflour white, the hyphae of Aspergilli are observed as hyaline, septate uniform width.Aspergilloma are treated with Amphotericin B

11.7. UNIT END EXERCISES

Two Mark Questions

Name the fungi that producing Mycotoxin.
Give examples for dimorphic fungus.
What is aspergilloma
What is germ tube.
What is candidsiasis
What is Gilchrist disease

Five Mark Questions

Explain lab diagnosis of Coccidioidomycoses
Describe pathogenesis of Histoplasmosis
Explain types Blastomycoses
Give a brief note on Paracoccidioides braciliensis.
Write a short account on candidiasis.
Describe Aspergillosis
How do you diagnose Cryptococcosis
Explain mycotoxicosis

Ten Mark Questions

Explain treatment and diagnosis of systemic mycoses.
Describe Candidiasis
Write a detailed note on cryptococcosis
Write an essay on systemic Mycoses

11.8. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

11.1. Fungi that exist inboth mycelia and yeast form at different environment are called dimorphic fungi.
11.2. It is a chemical extracted from Sperule.

11.3. Amphotericin B
11.4. Diseases caused by Candida albicans are called candidiasis.
11.5. Respiratory aspergillosis is called aspergilloma
11.6. Blood borne mycoses are called systemic mycoses.
11.7. Histoplasmosis is also called Darlings diseases.
11.8. Carcinogenic Toxin produced by Aspergillus are called Aflatoxin.
11.9. Diseses due to mycotoxin are called mycotoxicosis.
11.10. Toxin which affect nerve cells are called neurotoxin

11.9. SUGGESTED READINGS

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BLOCK-4 PARASITES, NEWLY EMERGED DISEASES &CONTROL MECHANISM

UNIT - XII PROTOZOOLOGY

Summary

12.1 INTRODUCTION

Medical parasitology traditionally has included the study of three major groups of animals. They are parasitic protozoa, parasitic helminths (worms)and those arthropods that directly cause disease or act as vectors of various pathogens. A parasite is a pathogen that simultaneously injures and derives sustenance from its host. In this part pathogenic protozoan diseases are described.

12.2 OBJECTIVES

After reading this chapter learners will be able to understand

Diseases of protozoa

Causative nature, pathogenesis, lab diagnosis and treatment of Amoebiosis and Malaria.

12.3. AMOEBIOSIS

12.3.1. Introduction

Amoebiosis is one of the intestinal disorder, which affect 10% of all the world population. Annual deaths are estimated between 40,000 and 100,000. Amebiosis is caused by *Entamoeba histolytica.*

12.3.2. Systemic Position

Phylum	-	Protozoa
Sub phylum	-	Sarcomastigophora
Superclass	-	Sarcodina
Class	-	Rhizopoda
Sub class	-	Lobosia
Order	-	Amoebida
Genus	-	Entamoeba
Species	-	histolytica.

Amoebas are unicellular organisms common in the environment, many are parasites of vertebrates and invertebrates. Relatively few species inhabit the human intestine and only *Entamoeba histolytica* is identified as a human intestinal pathogen.

12.3.3. Causative agent

Distribution - Worldwide distribution. Commonly available in Tropical, Subtropical, Temporate regions. Infection rate is higher in rural and densely populated areas.

Habitat – Endoparasite, Present in man and other mammals, Alive in mucous layer of colon

Habit - Feeds dissolved tissues, bacteria and RBCs, Causes fatal and serious disease. Infected individual discharge mucous and blood in their stool

Structure

E. histolytica has a relatively simple life cycle that alternates between Trophozoite and Cyst stages.

Trophozoite

The Trophozoite is the actively metabolizing, mobile stageand the cyst is dormant and environmentally resistant. Trophozoites vary remarkably in size-from 10 to 60 µm or more in diameter. When they are alive they may be actively motile (Unidirectional motility). Amoebas are anaerobic organisms and do not have Mitochondria. The finely granular endoplasm contains the nucleus and food vacuoles, which in turn may contain bacteria or red blood cells. The parasite is sheathed by a clear outer ectoplasm. Nuclear morphology is best seen in permanent stained preparations. The nucleus has a distinctive central karyosome and a rim of finely beaded chromatin lining the nuclear membrane. Finger like Pseudopodia is available.

Cyst

The cyst is a spherical structure, 10-20 µm in diameter, with a thin transparent wall.Fully mature cysts contain four nuclei with the characteristic amebic morphology. Rod-like structures (chromatoidal bars) are present variably, but are more common in immature cysts. Inclusions in the form of glycogen masses also may be present.

12.3.4. Clinical Manifestations

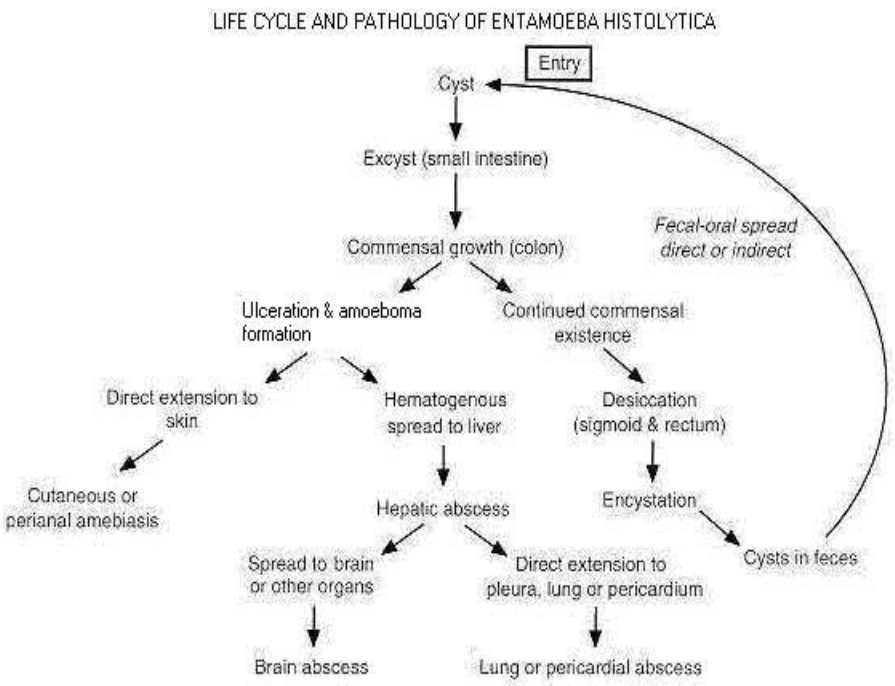
Fever, Amoebic dysentery -fulminant ulceration , Non dysentery gastroenteritis, Amoeboma formation, Amoebic colitis, Hepatomegaly, Amoebic abscess, Visceral amoebiosis

12.3.5. Multiplication, Life Cycle and Pathogenesis of E. histolytica.

Encystment occurs apparently in response to desiccation as the amoeba is carried through the colon. After encystment, the nucleus divides twice to produce a quadrinucleate mature cyst. Encysted quadrinucleate cysts of *E histolytica is ingested through* fecal-oral transmission or food and water. Ingested cysts of *E histolytica* excyst in the small intestine . Trophozoites are carried to the colon, where they mature and reproduce. Excystment occurs after ingestion and is followed by rapid cell division to produce four amoebas which undergo a second division. Each cyst thus yields eight tiny amoebas (Trophozoites).

Quadrinucleated Amoeba adheres to the colonic mucosal cells. The amoeba adherence molecule has been identified as a lectin, which can bind to either of two common carbohydrate components of cell membrane, galactose and *N*-acetyl glactoseamine. Amoeba attacks and kills the host cell. This cytolytic event is a result of incorporation in the host cell membrane of an amoeba-produced, pore-forming protein, **amoebapore.** This protein forms ion channels in lipid cell membranes and results in cell death within minutes of cell contact with the amoeba.

The initial lesion is in colonic mucosa, most often in the cecum or sigmoid colon. The slow transit of the intestinal contents in these two locations seems an important factor in invasion of the mucosa, both because it affords the amoeba greater mucosal contact time and because it permits changes in the intestinal milieu that may facilitate invasion. The initial superficial ulcer may deepen into the submucosa and muscularis to become the characteristic flask-shaped, chronic amoebic ulcer. Spread may occur by direct extension, by undermining of the surrounding mucosa until it sloughs, or by penetration that can lead to perforation or fistulous communication to other organs or the skin. If the amoebas gain access to the vascular or lymphatic circulation, metastases may occur first to the liver and then by direct extension or further metastasis to other organs, including the brain. If amoebas pass down the colon they encyst under the stimulus of desiccationand then are evacuated with the stool.



12.3.6. Epidemiology

Fecal-oral transmission occurs when food preparation is not sanitary or when drinking water is contaminated. Contamination may come directly from infected food handlers or indirectly from faulty sewage disposal.

12.3.7. Lab Diagnosis

Sample – Stool, Aspirates from intestinal and other organs, Exudates, Biopsy materials, Mucous from rectal ulcer

Microscopy - Saline and Iodine Wetmount & Concentration methods

The microscopic examination of direct smear has several purposes. They are To assess the worm burden of a patient. To provide quick diagnosis of a heavily infected specimen. To check organisms motility.

Serology - Serological methods include Gel diffusion, Immunoelectrophoresis, Countercurrent electrophoresis, Indirect Hemagglutination, Indirect Fluorescent Antibody, Skin tests, Enzyme-Linked Immuno Sorbent Assay (ELISA) and Latex Agglutination.

12.3.8. Control

Preventive measures are limited to environmental and personal hygiene. Treatment depends on drug therapy, Acute intestinal disease is best treated with Metronidazole at a dose of 750 mg three times a day orally for 10 day. In children the dose is 40 mg/kg/day divided into three doses and given orally for 10 days. Iodoquinol at an adult dose of 650 mg orally three times daily for 20 days or Diloxanide furoate at an adult dose of 500 mg orally three times daily for 10 days. Amoebic liver abscess is

best treated with Metronidazole, Dihydroemetin, Chloroquine or Dehydroemetine.

Check Your progress

12.1. What is amoeboma
12.2. What is cyst
12.3. Name the microscopicmethod to detect protozoa
12.4. Amoeboma
12.5. What is quadrinucleated cyst

12.4. MALARIA

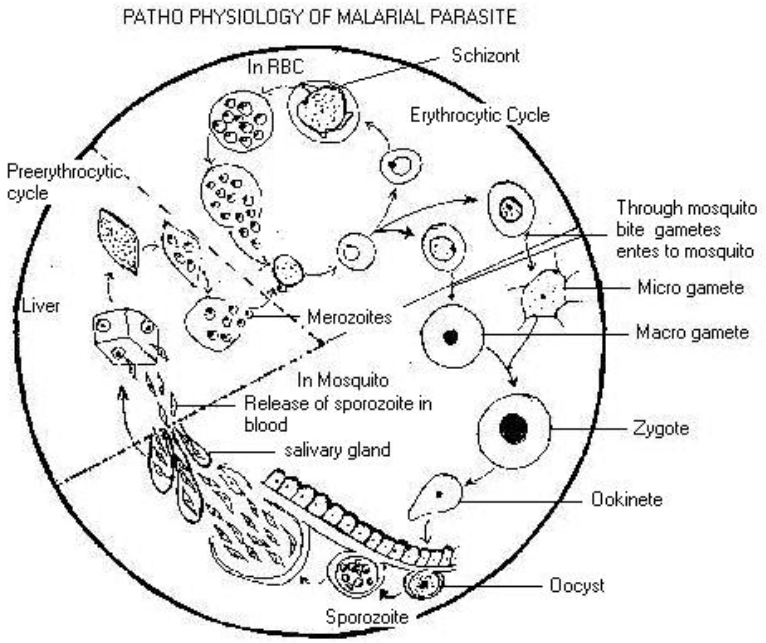
12.4.1. Introduction

Malaria has been a major disease of humankind for thousands of years. Malaria is caused by protozoa of the genus *Plasmodium.*

12.4.2. Causative agent

Four species cause disease in humans: *P falciparum, P vivax, P ovale* and *P malariae.* Malaria is spread to humans by the bite of female mosquitoes of the genus *Anopheles*. *P falciparum* and *P vivax* account for the vast majority of cases. *P falciparum* causes the most severe disease.

12.4.3. Structure And Life Cycle



Plasmodia pass through a number of stages in the course of their two host life cycle. The stage infective for humans is the uninucleate, lancet shaped sporozoite (approximately 1 X 7 µm). Sporozoites are produced by sexual reproduction in the midgut of vector anophelies mosquitoes and migrate to the salivary gland. When an infected *Anopheles* mosquito bites a human, she may inject sporozoites along with saliva into small blood vessels. Sporozoites are thought to enter liver

parenchymal cells within 30 minutes of inoculation. In the liver cell, the parasite develops into a spherical, multinucleate liverstage schizont which contains 2,000 to 40,000 uninucleate merozoites. This process of enormous amplification is called Exoerythrocytic Schizogony. This Exoerythrocytic or liver phase of the disease usually takes between 5 and 21 days, depending on the species of *Plasmodium*. However, in *P vivax* and *P ovale* infections, maturation of liverstage schizonts may be delayed for as long as 1 to 2 years. These liver-phase parasites are called hypnozoites.

The mature schizonts eventually rupture, releasing thousands of uninucleated merozoites into the bloodstream. Each merozoite can infect a Red Blood Cell. Within the red cell, the merozoite develops to form either an erythrocytic stage (blood-stage) schizont (by the process of erythrocytic schizogony) or a spherical or bananashaped, uninucleate gametocyte. The mature erythrocytic stage schizont contains 8 to 36 merozoites, each 5 to 10 µm long, which are released into the blood when the schizont ruptures. These merozoites proceed to infect another generation of erythrocytes.

The gametocyte, which is the sexual stage of the *Plasmodium*, is infectious for mosquitoes that ingest it while feeding. Within the mosquito, gametocytes develop into female and male gametes (macrogametes and microgametes, respectively), which undergo fertilization and then develop over 2 to 3 weeks into sporozoites that can infect humans. The delay between infection of a mosquito and maturation of sporozoites means that female mosquitoes must live a minimum of 2 to 3 weeks to be able to transmit Malaria. This fact is important in Malaria control efforts.

12.4.4. Pathogenesis

Clinical illness is caused by the Erythrocytic stage of the parasite. No disease is associated with sporozoites, the developing liver stage of the parasite, the merozoites released from the liver, or gametocytes. The first symptoms and signs of Malaria are associated with the rupture of erythrocytes when erythrocytic stage schizonts mature. This release of parasite material presumably triggers a host immune response. The cytokines, reactive oxygen intermediatesand other cellular products released during the immune response play a prominent role in pathogenesisand are probably responsible for the fever, chills, sweats, weaknessand other systemic symptoms associated with malaria. In the case of Falciparum Malaria (the form that causes most deaths), infected erythrocytes adhere to the endothelium of capillaries and postcapillary venules, leading to obstruction of the microcirculation and local tissue anoxia. In the brain this causes cerebral malaria; in the kidneys it may cause acute tubular necrosis and renal failure; and in the intestines it can cause ischemia (deficiency of blood) and ulceration, leading to gastrointestinal bleeding and to bacteremia secondary to the entry of intestinal bacteria into the systemic circulation.

12.4.5. Symptoms

The most characteristic symptom of Malaria is fever. Other common symptoms include Chills, Headache, Myalgias, Nauseaand Vomiting. Diarrhoea, Abdominal painand Cough are occasionally seen.

The first is a 15-to-60 minute cold stage characterized by shivering and a feeling of cold.

Second comes the 2-to-6 hour hot stage, in which there is fever, sometimes reaching 41°C, flushed, dry skinand often headache, nauseaand vomiting.

Finally, there is the 2-to-4 hour sweating stage during which the fever drops rapidly and the patient sweats.

In all types of Malaria the periodic febrile response is caused by rupture of mature schizonts. In *P vivax* and *P ovale* malaria, a brood of schizonts matures every 48 hours, so the periodicity of fever is Tertian ("tertian malaria"), whereas in *P malariae* disease, fever occurs every 72 hours ("quartan malaria"). The fever in Falciparum Malaria may occur every 48 hours, but is usually irregular, showing no distinct periodicity.

12.4.6. Lab Diagnosis

Diagnosis of Malaria generally requires direct observation of Malaria parasites in Giemsa-stained thick and thin blood smears. Thick blood smears are more difficult to interpret than thin blood smears but they are much more sensitive, as more blood is examined. Thin blood smears, in which parasites are seen within erythrocytes, are used to determine the species of the infecting parasite.

New diagnostic methods include a rapid antigen-capture dip stick test and a technique for detecting parasites with a fluorescent stain. Both of these tests are fast, easy to perform and are highly sensitive and specific

Other diagnostic methods include assays to detect Malaria antibodies and antigensand Polymerase Chain Reaction/DNA and RNA probe techniques. These techniques are used primarily in epidemiologic studies and immunization trials and rarely in the diagnosis of individual patients.

12.4.7. Control

Drugs used for treatment are Primaquine, Chloroquine, Mefloquine, Quinine, Quinidine, Pyrimethamine-sulfadoxine, Doxycycline, Halofantrine, Artemisnin, Proguanin

12.4.8. Prevention of Malaria

Individuals should avoid contact with the mosquito by wearing protective clothing. Use an insect repellent containing N,N-diethyl *m*toluamide (DEET). Sleeping under insecticide-impregnated bednets. For most of travelers, Mefloquine is the drug of choice and doxycycline is as acceptable alternative. Chloroquine plus Proguanil is another possible regimen for chloroquine-resistant areas, but this regimen is much less effective than Mefloquine or Doxycycline. Prophylaxis with Chloroquine or Mefloquine should begin 2 weeks before entering the Malarious area.

Check Your progress

12.6. What is sporozoite
12.7. What is Merozoite
12.8. What id Tertian Malaria
12.9. Quartan Malaria
12.10. Name stain used for malaria diagnosis

12.5. LET US SUM UP

Amoebiosis is one of the intestinal disorder, caused by *Entamoeba histolytica*. It is identified as a human intestinal pathogen. *E. histolytica* has a relatively simple life cycle that alternates between Trophozoite and Cyst stages. Fever, Amebic dysentery -fulminant ulceration , Non dysentery gastroenteritis, Amoeboma formation, Amoebic colitis, Hepatomegaly, Amoebic abscess, Visceral amoebiosis. Encystment occurs apparently in response to desiccation as the amoeba is carried through the colon. After encystment, the nucleus divides twice to produce a quadrinucleate mature cyst. Encysted quadrinucleate cysts of *E histolytica is ingested through* fecal-oral transmission or food and water. Ingested cysts of *E histolytica* excyst in the small intestine . Trophozoites are carried to the colon, where they mature and reproduce. Excystment occurs after ingestion and is followed by rapid cell division to produce four amoebas which undergo a second division. Each cyst thus yields eight tiny amoebas (Trophozoites). Preventive measures are limited to environmental and personal hygiene. Treatment depends on drug therapy, Acute intestinal disease is best treated with Metronidazole at a dose of 750 mg three times a day orally for 10 day.

Malaria has been a major disease of humankind for thousands of years. Malaria is caused by protozoa of the genus *Plasmodium.* Four species cause disease in humans: *P falciparum, P vivax, P ovale* and *P malariae.* Malaria is spread to humans by the bite of female mosquitoes of the genus *Anopheles*. Clinical illness is caused by the Erythrocytic stage of the parasite. No disease is associated with sporozoites, the developing liver stage of the parasite, the merozoites released from the liver, or gametocytes. The most characteristic symptom of Malaria is fever. Other common symptoms include Chills, Headache, Myalgias, Nauseaand Vomiting. Diarrhoea, Abdominal painand Cough are occasionally seen. The first is a 15-to-60 minute cold stage characterized by shivering and a feeling of cold. Second comes the 2-to-6 hour hot stage, in which there is fever, sometimes reaching 41°C, flushed, dry skinand often headache, nauseaand vomiting. Finally, there is the 2-to-4 hour sweating stage during which the fever drops rapidly and the patient sweats. Diagnosis of Malaria generally requires direct observation of Malaria parasites in Giemsa-stained thick and thin blood smears. Drugs used for treatment are Primaquine, Chloroquine, Mefloquine, Quinine, Quinidine, Pyrimethamine-sulfadoxine, Doxycycline, Halofantrine, Artemisnin, Proguanin

12.6. UNIT END EXERCISES

Two Mark Questions

What is Trophozote
Mention importance of Entamoeba cyst.
What is merozoite
What is sporozoite
Where thick and thin blood smear is used.
Name the stain used for staining Malarial Parasite
Plasmodium
Amoebapore

Five Mark Questions

Describe *Entamoeba histolytica* characters.
Give the life cycle of *Entamoeba histolytica*
Explain exoerythrocytic lifecycle of *Plasmodium*
Give a brief note on erythrocytic cycle of Plasmodium

Ten Mark Questions

Write an essay on Amoebiasis
Give a detailed note on malaria

12.7. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

12.1. Amoeboma is a major complication of sigmoid colon due to enatmoeba feeding on tissue. This leads to formation of flash like ulcer.
12.2. Cyst is a dormat form of protozoa.
12.3. Saline, iodine wetmout and Giemsa staining
12.4. Quadrinucleated cyst is a fully matured cyst which is converted to four trophozoites.
12.5. Sporozoite is a infective form of Malarial parasite, it is found in proboscis of Mosquito.
12.6. Merozoite is released during exoerythrocytic life cycle of Malarial parasite. It infects RBC.
12.7. In *P vivax* and *P ovale* malaria, a brood of schizonts matures every 48 hours, so the periodicity of fever is Tertian ("tertian malaria").
12.8. In *P malariae*, a brood of schizonts matures every 72 hours, so the periodicity of fever is quartan malaria.
12.9. Giemsa stain

12.8. SUGGESTED READINGS

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UNIT-XIII ANTIMICROBIAL SUBSTANCES

Summary

3.1 INTRODUCTION

The earliest evidence of successful chemotherapy is from ancient Peru, where the Indians used bark from the cinchona tree to treat Malaria. Paul Ehrlich is called father of Chemotherapy. He discovered p-rosaniline, which has antitrypanosomal effectsand arsphenamine, which is effective against Syphillis. Ehrlich postulated that it would be possible to find chemicals that were selectively toxic for parasites but not toxic to humans are called the "magic bullet". Penicillin G was discovered in 1929 by Fleming, In 1939 Florey and colleagues at Oxford University tested antimicrobial activities of lysozyme and again isolated Penicillin.

3.2 OBJECTIVES

After reading this unit reader will be able to understand mode of action of following antibacterial (Penicillin), antiviral (Amantidine), antifungal (Amphotericin) and antiparasitic drugs (Quinine and Metraindazole) antimicrobial agents.

3.3. BIOCHEMICAL BASIS OF ANTIMICROBIAL ACTION

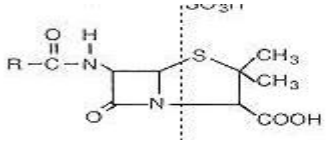
Bacterial cells grow and divide, replicating repeatedly to reach large numbers. To grow and divide, organisms must synthesize or take up many types of biomolecules. Antimicrobial agents interfere with specific processes that are essential for growth and/or division. Based on the mode of action antimicrobials are classified into

Inhibitors of bacterial and fungal cell walls,
Inhibitors of cytoplasmic membranes,
Inhibitors of nucleic acid synthesisand
Inhibitors of ribosome function.

Antimicrobial agents may be either bactericidal i.e., killing the target bacterium or bacteriostatic, i.e., inhibiting its growth.

3.4. ANTIBACTERIAL ANTIBIOTICS PENICILLIN

Penicillin is an antibacterial antibiotics. It was discovered in 1928 by Scottish scientist Alexander Flemming. This medicin is used for treatment since 1942. Gram-positive bacterial cell wall contains peptidoglycan and teichoic acidand the bacterium may or may not be surrounded by a protein or polysaccharide envelope. Gram-negative bacterial cell wall contain peptidoglycan, lipopolysaccharide, lipoprotein, phospholipidand protein. Penicillin kills susceptible bacteria by specifically inhibiting the transpeptidase that catalyzes the final step in cell wall biosynthesis, the cross-linking of peptidoglycan. Penicillin is a structural analog of the acyl-D-alanyl-D-alanine terminus of the pentapeptide side chains of nascent peptidoglycan. The critical attack site of anti-cell-wall agents is the peptidoglycan layer. This layer is essential for the survival of bacteria in hypotonic environments; loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death.



Penicillin

Penicillins are widely used to inhibit both Gram-positive and Gram-negative bacilli.

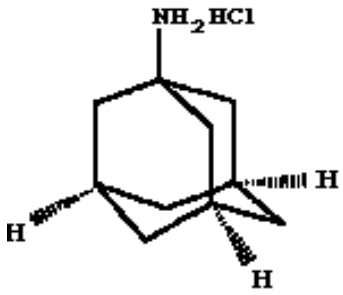
Penicillin is a group of antibiotics. There are different types of Penicillin they are Penicillin G (IV), Penicillin V (Oral) , Procaine Penicillin and Benzanthine Penicillin (IM).they are beta lactam group of antibiotic. It is a first discovered antibiotic. About 10% of peoples are allergic to penicillin.

Natural penicillins are Penicillin G, Penicillin K, Penicillin N, Penicillin O, Penicillin V, Semisynthetic and synthetic benicillin are as follows Methicillin, Nafcillin,Oxacillin, Cloxacillin, Dicloxacillin, Flucloxacillin,Ampicillin, Amoxicillin, Pivampicillin, Hetacillin, Bacampicillin, Metampicillin, Talampicillin, Epicillin,Carbenicillin,Ticarcillin, Temocillin,

13.5. ANTIVIRAL (AMANTIDINE)

Antiviral drugs are Categorized according to their point of action in viral replication cycle. Amantadine is a antiviral compound **that block attachment of virus and uncoating of virus.** These compounds inhibits fusion of viral envelop with endosome membrane. They prevent release of nucleocapsid in to the cytoplasm.They are used in treatment of influenza A infections. They act by binding to M2 protein.

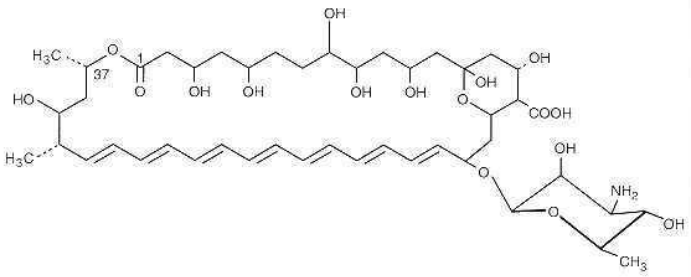
Amantadine:



13.6. ANTIFUNGAL (AMPHOTERICIN)

The polyene antibiotics act on sterol membrane by binding to rigid hydrophobic center and a flexible hydrophilic section. Polyene interact with fungal cells to produce a membrane-polyene complex that alters the membrane permeability, resulting in internal acidification of the fungus with exchange of K+ and sugars; loss of phosphate esters, organic acids, nucleotides; and eventual leakage of cell protein. Amphotericin B is used

Amphotericin B



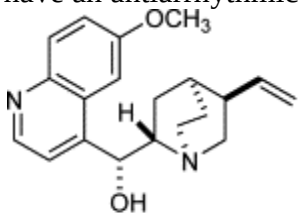
systemically

Amphotericin B is an antifungal medication used for serious fungal infections and leishmaniasis. The fungal infections it is used to treat include aspergillosis, blastomycosis, candidiasis, coccidioidomycosisand cryptococcosis. Amphotericin B was isolated from Streptomyces nodosus in 1955 and came into medical use in 1958. It is a essential medicine.

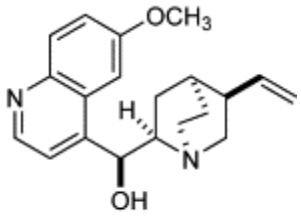
It is considered first line therapy for invasive mucormycosis infections, cryptococcal meningitis and certain Aspergillus and Candidial infections. Amphotericin B alone is insoluble in normal saline at a pH of 7. Therefore, several formulations have been devised to improve its intravenous bioavailability. Lipid-based formulations of amphoteracin B are no more effective than conventional formulations, although there is some evidence that lipid-based formulations may be better tolerated by patients and may have fewer adverse effects. Amphotericin B binds with ergosterol, a component of fungal cell membranes, forming pores that cause rapid leakage of monovalent ions (K+, Na+, H+ and Cl−) and subsequent fungal cell death

13.7. ANTIPARASITIC DRUGS (QUININE AND METRAINDAZOLE).

Quinine was first extracted from the bark of the South American cinchona tree. In 1944, the total synthesis of quinine was achieved by Woodward and Doering. Quinine exhibits specific toxicity against Plasmodium and has antipyeretic (fever-reducing) activity. Therefore, it has long been used as an antimalarial drug. Although many other antimalarial drugs such as chloroquinine have been developed based on the structure of quinine, it is still widely used since it is the sole compound to which Plasmodium has no resistance. Before 1820, the bark was first dried, ground to a fine powderand then mixed into a liquid (commonly wine), which was then drunk. Quinine is a flavor component of tonic water, bitter lemon, vermouthand cocktails. In the United States, the FDA limits quinine in tonic water to 83 ppm. Quinine is used as a standard substance for a bitter taste. Quinine, a diastereomer of quinine, is also extracted from the bark and elicits a bitter taste. It is reported to have an antiarrhythmic effect.



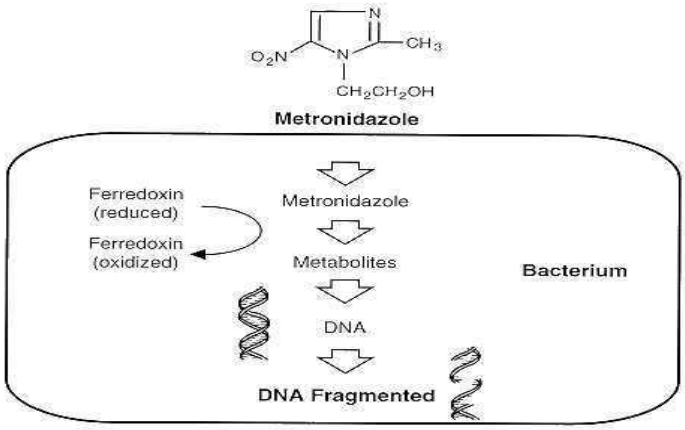
Quinine



Quinidine

Quinine, a cinchona alkaloid, was the first commercially available antimalarial agent; its use was supplanted when chloroquine became available. Well known for its antimalarial effects, quinine also is effective when used in combination with clindamycin for treatment of babesiosis. The use of this agent has grown with the spread of chloroquine resistanceand now there are increasing reports of clinical resistance to quinine in multiple geographic regions.

Oral quinine sulfate, in combination with pyrimethamine-sulfadoxine, tetracycline, or clindamycin, is the recommended therapy for P. falciparum malaria (falciparum malaria) acquired in areas of chloroquine resistance. A number of substances bind to DNA by intercalation. chloroquine inhibit plasmodia and schistosomes, respectively by inhibiting nucleic acid synthesis.



Metronidazole binds to DNAand cause DNA breakage. Metronidazole is an antibiotic that is used to treat a wide variety of infections. It works by stopping the growth of certain bacteria and parasites. Metrogel (topical metronidazole) is also used to treat rosacea, a skin condition. Vaginal metronidazole gel is also used to treat bacterial infections of the vagina.

Check Your Progress

13.1. How Penicillin inhibits gram positive bacteria.
13.2. Mention the use of Amantidine.
13.3. Give the uses of metronidazole

13.8. LET US SUM UP

.Antimicrobial agents interfere with specific processes that are essential for growth and/or division. Penicillin is an antibacterial antibiotics. It was discovered by Alexander Flemming in 1929. Gram-positive bacterial cell wall contains peptidoglycan and teichoic acidand the bacterium may or may not be surrounded by a protein or polysaccharide envelope. Penicillins are widely used to inhibit both Gram-positive and Gram-negative bacilli. Antiviral drugs are Categorized according to their point of action in viral replication cycle. Amantadine is a antiviral compound that block attachment of virus and uncoating of virus.

The polyene antibiotics act on sterol membrane by binding to rigid hydrophobic center and a flexible hydrophilic section. Amphotericin B is an antifungal medication used for serious fungal infections and leishmaniasis.

Quinine was first extracted from the bark of the South Americancinchona tree and isolated. Quinine is one of the most common drugs associated with Malaria. Metronidazole binds to DNAand cause DNA breakage

13.9. UNIT END EXERCISES

Two Mark Questions

Amphoterizin B
Penicillin G
Metronidazole

Five Mark Questions

Give the mode of action of Penicilln

Descibe Amantadine

Ten Mark Questions

Write a detailed note of antimicrobial substances

13.10. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

13.1. Penicillin inhibits gram positive bacteria by interfereing PG layer..

13.2. Amantidine is a antiviral drug.

13.3. Metronidazole is used for the treatment of protozoan infection. It interferes nucleic acid metabolism.

13.11. SUGGESTED READINGS

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UNIT XIV EMERGING AND RE-EMERGING INFECTIONS

Summary

14.1 INTRODUCTION

Emerging infections are recently appeared within a defined population. Emerging diseases are previously undetected or unknown infectious agents. Varients of previously described viruses are called reemerging viruses and diseases associated with these organisms are called emerging and reemerging diseases. Reemerging viruses are mutants of known viruses that cause new epidemics with considereable virulence. For example mutant of Influenza virus causes Swine flue H1N1. Peoples may unaware the nature of these viruses and experiences problem in diagnosis, prevention and treatment.

14.2 OBJECTIVES

After reading this unit readers will be able to understand

Emerging viruses
Reemerging viuses
Causative agents, pathogenesis, transmission, diagnosis and treatment of chikungunya, zika virus, h1n1 and ebolavius
Lab diagnosis of emerging and re- emerging infections.
Prevention of infectious diseases.

14.3. CHICKUNGUNYA

14.3.1. Introduction

Chikungunya is a viral disease. Chikungunya was first identified in Tanzania in the early 1952 and has caused periodic outbreaks in Asia and Africa since the 1960s. In India first major outbreakis noted in 1963 followed by in 2013. This disease is transmitted to humans by infected *Aedes aegypti* and *Aedes albopictus,* hence it is in Arbo viruses.

14.3.2. Causative agent

It is caued by chikunguny virus, which is the member of the genus *Alphavirus*, family togaviridae. It is an enveloped virus with icosahedral capsid. It contains positive sense single stranded RNA virus. The virus consists of four nonstructural proteins and three structural proteins. The structural proteins are the capsid and envelope spike proteins namely E1 and E2.

14.3.3. Replication

Chikungunya virus is able to multiply in epithelial as well as endothelial cells, fibroblasts, macrophages. Viruses are highly cytopathic innature but susceptible to type-I and –II Interferons. The replication and propagation of viruses is dependent on entry into permissive cells. Alphavirus entry into cells is initiated by receptor-binding, followed by clathrin-mediated endocytosis. Fusion to endosomal membranes transports nucleocapsid (NC) into the cytoplasm, where RNA is released after disassembly. Genomic RNA is used for both translation of proteins from genomic and subgenomic (26S) RNAand transcription of nascent (+)RNA via a (−)RNA template. ncoating of alphavirus nucleocapsids occurs almost immediately (~1 minute) after their penetration into the cytoplasm. 60S ribosomal RNA interacts with the C protein, facilitating uncoating of the nucleocapsid and release of viral RNA for initiation of protein synthesis. Upon synthesis, the E2 glycoprotein precursor, PE2 (p62 in SFV)and E1 glycoproteins interact with each other (preferentially in *cis*) to form heterodimers. These heterodimer complexes are then transported from the endoplasmic reticulum to the cell surface via the Golgi complex. At a late stage of transport, the PE2 precursor is cleaved in its lumenal domain by host furin-like protease to generate mature E2 and E3 proteins. This cleavage induces a conformational change that weakens the E1-E2

interaction in the spike heterodimer, priming the fusion peptide for activation upon exposure to low pH. Interactions between the C protein and the cytoplasmic domain of the E2 protein drive the budding process, with E1-E2 heterodimers forming an envelope around nucleocapsid-like particles. Upon release from cells, virions acquire a membrane bilayer derived from the host cell plasma membrane.

14.3.4. Pathogenesis

Susceptible cells to chikungunia virus are human epithelial and endothelial cells, primary fibroblastsand monocyte-derived macrophages. After the first round of replication there is a host immune response, but the virus goes to the lymph nodes and then to other tissues via the circulatory system. Replication at other tissues leads to the viremic phase of the disease. Upon infection, CHIKV indirectly stimulates production of type I interferon (IFN) via activation of non-hematopoietic cells, including primarily fibroblasts, an action which is essential for clearing CHIKV from the body. CHIKV also appears to induce a signaling cascade by activating interferon promoter stimulator 1 (IPS-1), leading to the buildup of IRF3-dependent mRNAs while also blocking these mRNAs from encoding proteins.

CD8+ lymphocytes are found in skin rashes of acute patients, while CD4+ T-cells comprise the majority in synovial effusions of chronic patients. There is evidence that inflammation stemming from CHIKV infection has consequences for osteoblast and osteoclast proliferation and function, which may contribute to the effects of chronic CHIKV. Several of the cytokines associated with infection, such as TNF-α, IL-6and IL-1, also promote osteoclast activity and have been associated with osteoclastogenesis.

14.3.5. Symptoms

Symptoms usually begin 3–7 days after being bitten by an infected mosquito. The most common symptoms are fever and joint pain. Other symptoms may include headache, muscle pain, joint swelling, or rash. Chikungunya disease does not often result in death, but the symptoms can be severe and disabling. Most patients feel better within a week. In some people, the joint pain may persist for months.

14.3.6. Lab Diagnosis

The symptoms of chikungunya are similar to those of dengue and Zika. Definitive laboratory diagnosis can be accomplished through viral isolation, RT-PCR or serological diagnosis. RT-PCR using nested primer pairs is used to amplify several chikungunya-specific genes from whole blood, generating thousands to millions of copies of the genes in order to identify them. RT-PCR can also be used to quantify the viral load in the blood. Serological diagnosis requires a larger amount of blood than the other methodsand uses an ELISA assay to measure chikungunya-specific IgM levels in the blood serum.

14.3.7. Prevention

Mosquito control focuses on eliminating the standing water where mosquitos lay eggs and develope as larva; if elimination of the standing water is not possible, insecticides or biocontrol agents can be added. Methods of protection against contact with mosquitos include using insect repellents with substances such as DEET. Wearing bite-proof long sleeves and trousers also offers protectionand garments can be treated with pyrethroids, a class of insecticides that often has repellent properties.

14.3.8. Treatment

There is no vaccine to prevent or medicine to treat chikungunya virus. Treat the symptoms properly. Get plenty of rest. Drink fluids to prevent dehydration. Take medicine such as acetaminophen or paracetamol to reduce fever and pain. Do not take aspirin and other non-steroidal anti-inflammatory drugs (NSAIDS until dengue can be ruled out to reduce the risk of bleeding).

Check Your Progress

14.1. Why the name chikungunia is given to this disease

14.2. Why NSAIDS drugs are not used for chikungunia treatment

14.4. ZIKA VIRUS

14.4.1. Introduction

Zika virus is emerging virus which belongs to the family Flaviviridae. This virus is first isolated from monkeys of Ziika forest of Uganda in 1947, hence the name Zika virus. This virus is transmitted primarily by Aedes mosquitoes, which bite during the day. This virus is related to dengue, yellow fever and Japanese encephalitis virus. Zika virus is a mosquito-borne flavivirus. Outbreaks of Zika virus disease have been recorded in Africa, the Americas, Asia and the Pacific. The first recorded outbreak of Zika virus disease was reported from the Island of Yap in 2007. This virus is associated with Guillain-Barré syndrome.

The Flavivirus genus consists of more than 70 enveloped, positive-strand RNA viruses including yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV). Altogether flaviviruses affect hundreds of millions of individuals every year.

14.4.2. Causative agent

Zika virus is a enveloped virus. It has icosahedral capsid and nonsegmented positive sense single stranded RNA. This virus is closely related Yellow fever virus. It is a positive sense single-stranded RNA as genome. Genome is packaged in the capsid. The outer envelope is formed by envelope protein and is the protective antigen. It aids in entry of the virus into the inside of the cell. The genome also encodes several nonstructural proteins (NS1, NS2a, NS2b, NS3, N4a, NS4b, NS5). NS1

is produced as secretory form also. NS3 is a putative helicaseand NS5 is the viral polymerase.

14.4.3. Transmission

Zika virus is primarily transmitted by the bite of an infected mosquito from the *Aedes aegypti*. *Aedes* mosquitoes usually bite during the day, peaking during early morning and late afternoon/evening. Zika virus is also transmitted from mother to fetus during pregnancy. It is also transmitted via sexual contact, transfusion of blood and blood productsand organ transplantation.

14.4.4. Replication

The virus enters host cells by receptor-mediated endocytosis. The geneome positive-sense RNA genome gains entry into the cytoplasm by viral glycoprotein-mediated membrane fusion. Flavivirus replication begins when the genome is recognized as messenger RNA and translated by host cell machinery to yield a single polyprotein. The polyprotein is co- and post-translationally cleaved by viral and cellular proteases into 10 gene products. The structural proteins capsid (C), precursor membrane (prM/M) and envelope (E) are incorporated into the virion, whereas the non-structural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 serve to coordinate the intracellular aspects of virus replication, assembly and modulation of host defense mechanisms. NS1 is essential for virus replication and inhibition of complement-mediated immune response. NS3 contains serine protease, Nucleoside 5' triphosphatase (NTPase), RNA helicaseand 5' RNA triphosphatase (RTPase) activities, while NS2B serves as a cofactor for the protease activity of NS3. NS5 contains methyltransferase and RNA-dependent RNA polymerase (RdRp) domains required for genome replication and capping of nascent RNA. Three non-enzymatic, integral membrane proteins NS2A, NS4A and NS4B are poorly understood. NS2A is required for virus replication and assembly. NS4A induces membrane rearrangement and autophagy to enhance viral replication, whereas NS4B modulates host immune response by suppressing the α/β interferon signaling and the helicase activity of NS3.

14.4.5. Pathogenesis

Flavivirus pathogenesis ranges from mild illness such as fever, rash and joint pain, to more severe symptoms such as hemorrhagic fever and fatal encephalitis.

The incubation period of Zika virus disease is estimated to be 3–14 days. Symptoms are generally mild and include fever, rash, conjunctivitis, muscle and joint pain, malaise or headache. Symptoms typically last for 2–7 days. Most people with Zika virus infection do not develop symptoms.

An increased risk of neurologic complications is associated with Zika virus infection in adults and children, including Guillain-Barré syndrome, neuropathy and myelitis.

14.4.5. Lab Diagnosis

A diagnosis of Zika virus infection can only be confirmed by laboratory tests of blood or other body fluids, such as urine or semen.

14.4.6. Treatment

There is no treatment available for Zika virus infection or its associated diseases.People with symptoms such as fever, rash, or arthralgia should get plenty of rest, drink fluidsand treat pain and fever with common medicines.

14.4.7. Prevention

Protection against mosquito bites during the day and early evening is a key measure to prevent Zika virus infection. Personal protection measures include wearing clothing that covers as much of the body as possible; using physical barriers such as window screens and closed doors and windows; and applying insect repellent to skin or clothing that contains DEET, IR3535 or icaridin. No vaccine is yet available for the prevention or treatment of Zika virus infection.

Check Your Progress

14.3. is there any vaccine for Zika virus

14.4. Mention namalogy of Zika Virus

14.5. H1N1

Refer Swine flu

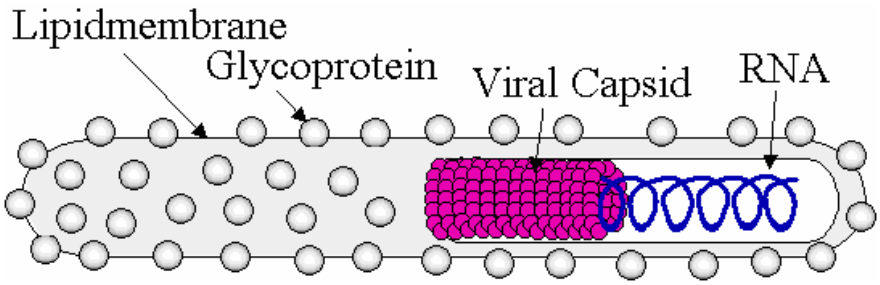
14.6. EBOLA

14.6.1. introduction

The Ebola virus causes an acute, serious illness which is often fatal. It is first appeared in 1976. Ebola virus disease (EVD), formerly known as Ebola haemorrhagic fever. The virus is transmitted to people from wild animals and spreads in the human population through human-to-human transmission. The average EVD case fatality rate is around 50%. Community engagement is key to successfully controlling outbreaks. Early supportive care with rehydration, symptomatic treatment improves survival. There is no licensed treatment proven to neutralize the virus but a range of blood, immunological and drug therapies are under development. The disease occurred first in a village near Ebola river, hence the name of the diseases Ebola.

14.6.2. Causative agent

Ebola virus is belongs to filo virus group. It is pleomorphic in nature. They are enveloped, filomentous or shorter U shaped of Circular thread like virus. Virus have helical nucleocapsid. It contains single stranded negative sense RNA as a genome. This virus is inactivated by heating at 60°C for 30 minutes. UV rays, gamma rays, lipid solvents, phenolic group of disinfectants are able to inactivate the virus. Ebola is a highly virulent virus.



14.6.3. Transmission

It is thought that fruit bats of the Pteropodidae family are natural Ebola virus hosts. Ebola is introduced into the human population through close contact with the blood, secretions, organs or other bodily fluids of infected animals such as fruit bats, chimpanzees, gorillas, monkeys, forest antelope or porcupines found ill or dead or in the rainforest. Ebola then spreads through human-to-human transmission via direct contact

14.6.4. Multiplication

Ebola viruses replicate efficiently in all cell types including endothelial cells, macrophages and parenchymal cells. Platelet dysfunction appears to be the major pathogenic factor of this disease. After incubation period vius induce a specific symptoms.

14.6.5. Symptoms

The incubation period is from 2 to 21 days. A person infected with Ebola cannot spread the disease until they develop symptoms. Symptoms of ebola are fever, fatigue, muscle pain, headache, sore throat, vomiting, diarrhoea, rash. In some cases, both internal and external bleeding .

14.6.6. Laboratory diagnosis

Low white blood cell and platelet counts, Elevated liver enzymes. Antibody-capture enzyme-linked immunosorbent assay (ELISA), Direct immunofluorescence test detects eosinophilic cytoplasmic inclusion bodies. Serum neutralization test. Reverse transcriptase polymerase chain reaction (RT-PCR) assay. Electron microscopy and ·virus isolation by cell culture.

14.6.7. Treatment

Supportive care - rehydration with oral or intravenous fluids - and treatment of specific symptoms improves survival. There is as yet no proven treatment available for EVD. However, a range of potential treatments including blood products, immune therapies and drug therapies are currently being evaluated.

14.6.9. Vaccines

An experimental Ebola vaccine proved highly protection against Ebola Virus Disease. The vaccine, called rVSV-ZEBOV is effective for ebola viral disease. Community involvement is a key factor in controlling the disease. .

Check Your Progress

14.5. What is the other name of Ebola

14.6. How ebola virus transmitted

NATIONAL PROGRAMME ON PREVENTION OF INFECTIOUS DISEASES

Infectious diseases remain a leading cause of morbidity, disabilityand mortality worldwide. To overcome problems various organizations play a vital role in prevention and control of infectious diseases.

Key Objectives of preventive programme are

- Enhance community awareness on infectious diseases and lay stress on preventive measures among general population especially high-risk groups and in hotspots.
- Provide early diagnosis and management of infectious diseases at all levels of healthcare
- Develop standard diagnostic and treatment protocols for management of infectious diseass and its complications.
- Strengthen the existing infrastructure facilities, build capacities of existing human resources and raise additional human resources, where required, for providing comprehensive services for management of infectious diseases and its complications in all districts of the country.
- Develop linkages with the existing National programs towards awareness, prevention, diagnosis and treatment for infectious diseases.

Components of Preventing Infectious Disease

1.Preventive component:

Awareness generation & behaviour change communication
Immunization to possible infectious diseases.
Safety of blood and blood products
Injection safety, safe socio-cultural practices
Safe drinking water, hygiene and sanitary toilets

2.Diagnosis and Treatment:

Free screening, diagnosis and treatment of infectious disease would be made available at all levels of health care in a phased manner.

Provision of linkages, including with private sector and not for profit institutions, for diagnosis and treatment.

Engagement with community/peer support to enhance and ensure adherence to treatment and demand generation.

3.Monitoring and Evaluation, Surveillance and Research

Effective linkages to the surveillance system would be established and operational research would be undertaken through Department of Health Research (DHR). Standardised monitoring & evaluation framework would be developed and an online web based system is established.

4.Training and Capacity Building:

This will be a continuous process and will be supported by NCDC (National Centre for Disease Control), ILBS (Institute of Liver and Biliary Sciences) and state tertiary care institutes

Example

National Viral Hepatitis Control Program (NVHCP)

The National Viral Hepatitis Control Program has been launched by Ministry of Health and Family Welfare, Government of India on the occasion of the World Hepatitis Day, 28th July 2018. It is an integrated initiative for the prevention and control of viral hepatitis in India to achieve Sustainable Development Goal (SDG) 3.3 which aims to ending viral hepatitis by 2030. This is a comprehensive plan covering the entire gamut from Hepatitis A, B, C, D & Eand the whole range from prevention, detection and treatment to mapping treatment outcomes. Operational Guidelines for National Viral Hepatitis Control Program, National Laboratory Guidelines for Viral Hepatitis Testing and National Guidelines for Diagnosis and Management of Viral Hepatitis were also released.

Check Your Progress

14.7. What is Infectious disease

14.8. What is the main aim of preventing infectious disease.

14.7. LET US SUM UP

Chikungunya is a viral disease caued by chikunguny virus. It is the member of the genus *Alphavirus*, family togaviridae. It is an enveloped virus with icosahedral capsid. It contains positive sense single stranded RNA virus. The most common symptoms are fever and joint pain. Other symptoms may include headache, muscle pain, joint swelling, or rash. Definitive laboratory diagnosis can be accomplished through viral isolation, RT-PCR, or serological diagnosis. RT-PCR using nested primer pairs is used to amplify several chikungunya-specific genes from whole blood, generating thousands to millions of copies of the genes in order to identify them. There is no vaccine to prevent or medicine to treat chikungunya virus. Treat the symptoms properly.

Zika virus is emerging virus which belongs to the family Flaviviridae. This virus is first isolated from monkeys of Ziika forest of Uganda in 1947, hence the name Zika virus. This vius is transmitted primarily by Aedes mosquitoes. Zika virus is a enveloped virus. It has icosahedral capsid and nonsegmented positive sense single stranded RNA. It is an enveloped virus. The incubation period of Zika virus disease is estimated to be 3–14 days. Symptoms are generally mild and include fever, rash, conjunctivitis, muscle and joint pain, malaise or headache. Symptoms typically last for 2–7 days. Most people with Zika virus infection do not develop symptoms. A diagnosis of Zika virus infection can only be confirmed by laboratory tests of blood or other body fluids, such as urine or semen. There is no treatment available for Zika virus infection or its associated diseases.

The **Ebola virus** causes an acute, serious illness which is often fatal. It is first appeared in 1976. Ebola virus disease (EVD), formerly

known as Ebola haemorrhagic fever. It contains single stranded negative sense RNA as a genome. Ebola viruses replicate efficiently in all cell types including endothelial cells, macrophages and parenchymal cells. Platelet dysfunction appears to be the major pathogenic factor of this disease. After incubation period vius induce a specific symptoms. The incubation period is from 2 to 21 days. A person infected with Ebola cannot spread the disease until they develop symptoms. Symptoms of ebola are fever, Fatigue, Muscle pain, Headache, Sore throat, Vomiting, Diarrhoea, Rash. In some cases, both internal and external bleeding . Low white blood cell and platelet counts, Elevated liver enzymes. Supportive care - rehydration with oral or intravenous fluids - and treatment of specific symptoms improves survival. The vaccine, called rVSV-ZEBOV is effective.

14.8. UNIT END EXERCISES

Two Mark Questions

How ebola virus transmitted
What is ebola haemorrhagic fever.
What are the modes of treatment for Ebola Virus.
Explain methods of Ebola diagnosis.
Mention IP of ebola
What is Aedes aegeyti
Explain the nature of Zika virus
Give the nature of Chikunguinea virus

Five Mark Questions

Explain diagnosis of Chikunguinea.
Describe symptoms of Ebola virus disease
How zika virus is treated and prevented.
What are the methods available to prevent infectious diseases.

Ten Mark Questions

Write an essay on Ebola Virus
Give a brief note on Chikunguinea
Describe Zika Virus.

14.9. ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

14.1. it is based on the city of Tanzania where chikungunia was firs originated.
14.2. NSAIDS drugs like brufen cause internal bleeding.
14.3. No
14.4. This virus is first isolated from Ziika forest of Uganda.
14.5. Ebola haemorrhagic fever
14.6. Through Mosquito
14.7Those diseses that are easily transmittable are called infectous disease.
14.8. Prevent transmission and controlling.

14.10. SUGGESTED READINGS

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