**Semester - II**

**I9MBP202 MEDICAL BACTERIOLOGY 4H – 4C**

**Instruction Hours / week: L: 4 T: 0 P: 0** **Marks:** Internal: **40** External: **60** Total: **100**

**End Semester Exam:** 3 Hours

**COURSE OBJECTIVES**

- Medical Bacteriology introduces basic principles and then applies clinical relevance of many etiological agents responsible for global infectious diseases.
- The infectious disease cycle of the pathogens enables to solve the epidemics. The territory covered by infections and the immune response expands each year;
- We focus on pathogenic mechanisms in order to foster a student's ability to solve problems in their future clinical career.

**COURSE OUTCOME**

- Demonstrate an understanding at an advanced level of microbial virulence mechanisms and host response to infection.
- Application of molecular techniques to medical microbiology; biochemical and genetic mechanisms of antimicrobial agent activity, microbial susceptibility and resistance to antimicrobial agents.
- Demonstrate an understanding of skin and respiratory tract infections (microbial causes, pathogenesis, transmission of infection, diagnosis, prevention and treatment) by being able to identify a unknown organisms in clinical samples, and describe the pathogenesis of important pathogens.

**UNIT I- Isolation and identification of pathogens**

Laboratory precaution and guidelines – Aseptic collection – transportation – handling and examination of pathological specimens – methods of isolation, identification and interpretation of pathogenic organisms – Antibiotic susceptibility testing

**UNIT II - Infections**

Infections – types – methods – infectious disease cycle. Definitions of Epidemics, Endemics Pandemics and investigation of epidemics and control. Definition of pathogens, Saprophytes and Commensals. Quality control in microbiology lab

**UNIT III - Gram positive organisms**

Morphology, cultural characteristics, antigenic property, pathogenecity, laboratory diagnosis and treatment.*Staphylococcus* sp.,*Streptococcus*sp., *Bacillus* sp., *Corynebacterium*sp., *Clostridium sp. Mycobacterium* sp.

**UNIT IV - Gram negative organisms**

Morphology, cultural characteristics, antigenic property, pathogenecity, laboratory diagnosis and treatment. *E.coli, Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Vibrio* sp., *Salmonella* sp., *Shigella*sp.,*Treponema*sp., *Leptospira*sp; *Neisseria* sp. and *Haemophilus*sp.

**UNIT – V – Infection and Therapy**

Nosocomial infection – Urinary tract infection, Respiratory tract infection, Sexually transmitted disease – Immunoprophylaxis – Antimicrobial chemotheraphy, Antibiotics, second line drugs.Vaccines.

## SUGGESTED READINGS

1. Ananthanarayanan, R., and Panicker, C.K.J., (2005). Text Book of Microbiology (7thed.). Orient Longman, NewDelhi.
2. Salle, A.J. (2008). Fundamentals principles of bacteriology. T.M.H. Ed.). McGrawHill.
3. Carl Fraenkel. (2012). Text book of bacteriology. Printing company publishers, NewYork.
4. Brook,G.F., J., Butel, S., Stephen, A., and Morse, A., (2003). Medical Microbiology, (22nded.). McGrawHill.
5. Chakraborty, P. (2003). A Text book of Microbiology. (2nded.). New Central Book Agency (P) Ltd., Calcutta.
6. Dismukes, W.E., Pappas, P.G., and Sobel, D., (2003). Clinical Mycology. Oxford University Press, UK.
7. Jawetz, E., Melnic, J.L., and Adelberg, E.A., (2001). Review of Medical Microbiology. (22nded.). Lange Medical Publishers.NY

# KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
**Coimbatore – 641 021.**
**(For the candidates admitted from 2017 onwards)**

## DEPARTMENT OF MICROBIOLOGY

**SUBJECT NAME: MEDICAL BACTERIOLOGY**
**SEMESTER: II**
**SUB.CODE:19MBP202** **CLASS: I M.Sc MB**

## LECTURE PLAN
## DEPARTMENT OF MICROBIOLOGY

| S.No | Lecture DurationPeriod | Topics to be Covered | Support Material/Page Nos |
|---|---|---|---|
| | | **UNIT-I** | |
| 1 | 1 | Laboratory precautions and guidelines | W1 |
| 2 | 1 | Aseptic collection | W2 |
| 3 | 1 | Aseptic collection | W2 |
| 4 | 1 | Transportation | T2:208 - 210 |
| 5 | 1 | Handling of pathological specimens | W3 |
| 6 | 1 | Methods of isolation | W3 |
| 7 | 1 | Identification and interpretation of pathogenic organism | W3 |
| 8 | 1 | Antibiotic susceptibility testing | T1:579-580,R1:884 |
| 9 | 1 | Antibiotic susceptibility testing | T1:579-580,R1:884 |
| 10 | 1 | Recapitulation and discussion of question | |
| | **Total No of Hours Planned For Unit 1=10** | | |
| | | **UNIT-II** | |
| 1 | 1 | Infections | T1: 59 - 64 |
| 2 | 1 | Types of infections | T1: 59 - 64 |
| 3 | 1 | Methods of infections – | R1: 852-856 |
| 4 | 1 | Infectious disease cycle | R1: 856-859 |
| 5 | 1 | Definitions of Epidemics, Endemics Pandemics | T1:64 - 69 |
| 6 | 1 | Investigation of epidemics and control | T1:64 - 69 |
| 7 | 1 | Definitions of pathogens, saprophytes and commensals | T1: 59 - 64 |
| 8 | 1 | Quality control in microbiology lab | T1: 33-42 |
| 9 | 1 | Recapitulation and discussion of question | |
| | **Total No of Hours Planned For Unit II=09** | | |

| | | | |
|---|---|---|---|
| | | **UNIT-III** | |
| 1 | 1 | Morphology, cultural characteristics, pathogenecity and laboratory diagnosis. *Staphylococcus Sp.,* | T1:177 – 182, R2: 199-205 |
| 2 | 1 | *Staphylococcus Sp.,* | T1:177 – 182, R2: 199-205 |
| 3 | 1 | *Streptococcus Sp.,* | T1: 187 – 192, R2:209 - 221 |
| 4 | 1 | *Bacillus Sp.,* | T1: 222 – 224, R2: 175-178 |
| 5 | 1 | *Corynebacterium Sp.,* | T1: 212-215, R2: 188-191 |
| 6 | 1 | *Clostridium Sp.,* | T1: 228-232,R2:178-183 |
| 7 | 1 | *Clostridium Sp.,* | T1: 228-232,R2:178-183 |
| 8 | 1 | *Mycobacterium Sp.,* | T1: 228-232,R2:178-182 |
| 9 | 1 | *Mycobacterium Sp.,* | T1: 228-232,R2:178-182 |
| 10 | 1 | Recapitulation and discussion of question | |
| | **Total No of Hours Planned For Unit III= 10** | | |
| | | **UNIT-IV** | |
| 1 | 1 | Morphology, cultural characteristics, antigenic property, pathogenecity, laboratory diagnosis and treatment. *E.coli* | T1: 251-257, R2:233-235 |
| 2 | 1 | *Klebsiella Sp., Proteus Sp.,* | T1: 258-261,R2:235 |
| 3 | 1 | *Pseudomonas Sp.,* | T1:294-297,R2:245-243 |
| 4 | 1 | *Vibrio Sp.,* | T1:281- 293,R2255-258 |
| 5 | 1 | *Salmonella Sp.,* | T1:267-280,R2: 238-240 |
| 6 | 1 | *Shigella Sp.,* | T1:262-266,R2: 236-237 |
| 7 | 1 | *Treponema Sp.,* | T1:350-358 |
| 8 | 1 | *Leptospira Sp.,* | T1: 212-214, R2: 296 |
| 9 | 1 | *Neiseiria Sp.,* | T1: 204-211, R2: 285-293 |
| 10 | 1 | *Haemophilus Sp.,* | T1: 306-311, R2: 265-268 |
| 11 | 1 | Recapitulation and discussion of question | |
| | **Total No of Hours Planned For Unit IV=11** | | |

| | | | |
|---|---|---|---|
| | | **UNIT-V** | |
| 1 | 1 | Nosocomial infection | T1: 552, 555, 583 |
| 2 | 1 | Urinary tract infection | T1: 552, 554, 582-583 |
| 3 | 1 | Respiratory tract infection | T1: 552, 582-583, 585 |
| 4 | 1 | Sexually transmitted diseases | T1: 552, 582-583, 585 |
| 5 | 1 | Immuno prophylaxix – Antimicrobial chemotherapy | T1: 581-582 |
| 6 | 1 | Antibiotics | T1: 583-585 |
| 7 | 1 | Second line drugs, Vaccines | T1: 432-435, T1: 586-589 |
| 8 | 1 | Recapitulation and discussion of question | |
| 9 | 1 | Old question paper discussion (Last Five years) | |
| 10 | 1 | Old question paper discussion (Last Five years) | |
| | **Total No of Hours Planned for unit V=10** | | |

**SUGGESTED READINGS:**


1. Ananthanarayanan, R. and C.K.J. Panicker, 2005. Text book of Microbiology. 7th Edition. Orient Longman. New Delhi.
2. Fritz, H.Kayser, Kurl-A Bienz, Johaanereckert, Rolf,M. Zinker nagel, MedicalMicrobiology, 2005, Thieme, New York.
3. Prescott, L.M., J.P. Harley and C.A. Klein, 2003. Microbiology, 5th Edition McGraw Hill Publishing Company Limited. New York.
4. Jawetz, E., J.L. Melnic and E.A. Adelberg, 2001. Review of Medical Microbiology. 22nd Edition. Lange Medical Publishers. NY.


**WEBSITES**

W1: www.chem.mtu.edu
W2: www.gosh.nhs.uk
W3: www.bounless.com

## UNIT-I
## SYLLABUS

Laboratory precaution and guidelines – Aseptic collection – transportation – handling and examination of pathological specimens – methods of isolation, identification and interpretation of pathogenic organisms – Antibiotic susceptibility testing.

**Laboratory safety precautions**

**Personal and General laboratory safety**

1. Never eat, drink, or smoke while working in the laboratory.
2. Read labels carefully.
3. Do not use any equipment unless you are trained and approved as a user by your supervisor.
4. Wear safety glasses or face shields when working with hazardous materials and/or equipment.
5. Wear gloves when using any hazardous or toxic agent.
6. Clothing: When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals should not be worn in the lab at any time. Shoes are required when working in the machine shops.
7. If you have long hair or loose clothes, make sure it is tied back or confined.
8. Keep the work area clear of all materials except those needed for your work. Coats should be hung in the hall or placed in a locker. Extra books, purses, etc. should be kept away from equipment that requires air flow or ventilation to prevent overheating.
9. Disposal - Students are responsible for the proper disposal of used material if any in appropriate containers.
10. Equipment Failure - If a piece of equipment fails while being used, report it immediately to your lab assistant or tutor. Never try to fix the problem yourself because you could harm yourself and others.
11. If leaving a lab unattended, turn off all ignition sources and lock the doors.
12. Never pipette anything by mouth.
13. Clean up your work area before leaving.
14. Wash hands before leaving the lab and before eating.

**Electrical safety**

1. Obtain permission before operating any high voltage equipment.
2. Maintain an unobstructed access to all electrical panels.
3. Wiring or other electrical modifications must be referred to the Electronics Shop or the Building Coordinator.
4. Avoid using extension cords whenever possible. If you must use one, obtain a heavy- duty one that is electrically grounded, with its own fuse, and install it safely. Extension cords should not go under doors, across aisles, be hung from the ceiling, or plugged into other extension cords.
5. Never, ever modify, attach or otherwise change any high voltage equipment.
6. Always make sure all capacitors are discharged (using a grounded cable with an insulating handle) before touching high voltage leads or the "inside" of any equipment even after it has been turned off. Capacitors can hold charge for many hours after the equipment has been turned off.
7. When you are adjusting any high voltage equipment or a laser which is powered with a high voltage supply, USE ONLY ONE HAND. Your other hand is best placed in a pocket or behind

your back. This procedure eliminates the possibility of an accident where high voltage current flows up one arm, through your chest, and down the other arm.

**Mechanical safety**

1. When using compressed air, uses only approved nozzles and never direct the air towards any person.
2. Guards on machinery must be in place during operation.
3. Exercise care when working with or near hydraulically- or pneumatically-driven equipment. Sudden or unexpected motion can inflict serious injury.

**Chemical safety**

1. Treat every chemical as if it were hazardous.
2. Make sure all chemicals are clearly and currently labeled with the substance name, concentration, date, and name of the individual responsible.
3. Never return chemicals to reagent bottles. (Try for the correct amount and share any excess.)
4. Comply with fire regulations concerning storage quantities, types of approved containers and cabinets, proper labeling, etc. If uncertain about regulations, contact the building coordinator.
5. Use volatile and flammable compounds only in a fume hood. Procedures that produce aerosols should be performed in a hood to prevent inhalation of hazardous material.
6. Never allow a solvent to come in contact with your skin. Always use gloves.
7. Never "smell" a solvent!! Read the label on the solvent bottle to identify its contents.
8. Dispose of waste and broken glassware in proper containers.
9. Clean up spills immediately.
10. Do not store food in laboratories.

**Lasers safety**

1. NEVER, EVER LOOK INTO ANY LASER BEAM, no matter how low power or "eye safe" you may think it is.
2. Always wear safety goggles if instructed by your Instructor or Teaching Assistant.
3. The most common injury using lasers is an eye injury resulting from scattered laser light reflected off of mountings, sides of mirrors or from the "shiny" surface of an optical table. The best way to avoid these injuries is to always wear your goggles and NEVER LOWER YOUR HEAD TO THE LEVEL OF THE LASER BEAM! The laser beam should always be at or below chest level.
4. Always use "beam stops" to intercept laser beams. Never allow them to propagate into the laboratory. Never walk through a laser beam. Some laser beams of only a few watts can burn a hole through a shirt in only a few seconds.
5. If you suspect that you have suffered an eye injury, notify your instructor or teaching assistant IMMEDIATELY! Your ability to recover from an eye injury decreases the longer you wait for treatment.

**Additional Safety Guidelines**

- Never do unauthorized experiments.
- Never work alone in laboratory.
- Keep your lab space clean and organized.
- Do not leave an on-going experiment unattended.
- Always inform your instructor if you break a thermometer. Do not clean mercury yourself!!
- Never taste anything. Never pipette by mouth; use a bulb.
- Never use open flames in laboratory unless instructed by TA.

- Check your glassware for cracks and chips each time you use it. Cracks could cause the glassware to fail during use and cause serious injury to you or lab mates.
- Maintain unobstructed access to all exits, fire extinguishers, electrical panels, emergency showers, and eye washes.
- Do not use corridors for storage or work areas.
- Do not store heavy items above table height. Any overhead storage of supplies on top of cabinets should be limited to lightweight items only. Also, remember that a 36" diameter area around all fire sprinkler heads must be kept clear at all times.
- Areas containing lasers, biohazards, radioisotopes, and carcinogens should be posted accordingly. However, do not post areas unnecessarily and be sure that the labels are removed when the hazards are no longer present.
- Be careful when lifting heavy objects. Only shop staff may operate forklifts or cranes.
- Clean your lab bench and equipment, and lock the door before you leave the laboratory.

**Specimen collection – microbiology**

**Introduction**

Microbiological and virological laboratory testing has a key role in the management of children with infection. Accurate and rapid identification of significant micro-organisms is vital for guiding optimal anti-microbial therapy, and improving outcome from infectious disease.

Laboratory diagnosis is also essential for effective infection control in both the hospital and community settings, as well as providing invaluable epidemiological data. Clinicians (including nurses, doctors and professionals allied to medicine) have responsibility for the collection and safe transportation of samples to the laboratory. The validity of test results largely depends on good practice in the "pre-test" stage and it is essential that documentation is accurate and comprehensive. Microbiological tests are not as standardised as some other lab tests; the way in which a sample is processed and the results are interpreted depend heavily on the information provided with the specimen.

Contamination of samples, especially those from normally sterile sites such as blood or cerebrospinal fluid, leads to misleading results, inappropriate antibiotic usage and unnecessary laboratory work. Prolonged periods of storage at ambient temperature and delay in transport of specimens to the laboratory may increase the number of contaminants present. It is therefore essential that every effort should be made to avoid these problems.

Note: While this guideline refers to the 'child' throughout, all activities are applicable to young people.

**Rationale for specimen collection**

Specimen collection is undertaken when laboratory investigation is required for the examination of material, eg tissue, body fluid or faeces to aid diagnosis.

**Preparation**

- Laboratory request forms are printed from the Patient Information Management System (PiMS). Use the labels on the form to label the specimen accompanying the form. These are bar coded to aid the audit trail.
- All specimens must be clearly labelled to identify their source.
- **DO NOT** pre-label specimen containers as this increases the risk of errors. The specimen must be labelled next to the child/patient when the sample is taken.
- A laboratory request form with the following information must accompany the specimen. This aids interpretation of results and reduces the risk of errors.
    1. Patient's name, DOB, ward/department and hospital number.
    2. Type of specimen and the site from which it was obtained.
    3. Date and time collected.

4. Diagnosis with history and reasons for request such as returning from abroad (specify country) with diarrhoea and vomiting, rash, pyrexia, catheters in situ or invasive devices used, or surgical details regarding post-operative wound infection.
5. The question that needs an answer by having the sample tested.
6. Any antimicrobial drug(s) given.
7. Consultant's name.
8. Name/bleep number of the clinician who ordered the investigation, as it maybe necessary to telephone preliminary results and discusses treatment before the final result is authorised.

- Always explain the procedure to the child and parent and the reasons for taking the specimen. Separate permission must be obtained from the child and parent if specimens are sought for research purposes. They have a right to refuse without any obligation (Ethics Advisory Committee 1992).
- Hands should be washed before and after specimen collection (see our hand hygiene clinical guideline). In line with standard precautions, appropriate personal protective equipment should be worn when collecting or handling specimens.
- If an infection is suspected, eg when a patient has respiratory symptoms or loose stools, the appropriate isolation precautions should be applied even before the results of the specimen are available. The isolation precautions should be based on the symptoms the child is presenting with. Once the result of the specimen is available, the need and type of the isolation precautions can be re-assessed according to the GOSH Standard and Isolation Precautions Policy (available to GOSH staff internally on the GOSHweb intranet site).
- When collecting certain specimens, eg catheter urines and cerebro-spinal fluid, every effort should be made to avoid infecting the child. An appropriate aseptic or aseptic non-touch technique should be used.
- All pathological specimens must be treated as potentially infectious and local written laboratory protocols should be followed for the safe handling and transportation of specimens (Health Services Advisory Committee 1986). Specimens should be collected in sterile containers with close fitting lids to avoid contamination and spillage. All specimen containers must be transported in a double-sided, self-sealing polythene bag with one compartment containing the laboratory request form and the other the specimen.
- Ideally microbiological specimens should be collected before beginning any treatment such as antibiotics or using antiseptics. However, treatment must not be delayed in serious sepsis.
- When collecting pus specimens obtain as much material as possible as this increases the chance of isolating micro-organisms which maybe difficult to grow or are minimal in number eg tuberculosis. Pus should be sent in a sterile specimen container, not on a swab.
- Transport medium may be used to preserve micro-organisms during transportation. Charcoal medium improves the isolation of bacteria by neutralising toxic substances such as naturally occurring fatty acids found on the skin.
- As many viruses do not survive well outside the body special viral transport medium is used. This is obtained from the Virology Department, Level 4 Camila Botnar Laboratory (CBL). It may be stored at room temperature on ward, but should only be used for viral investigation. The viral transport medium must not be used after the expiry date.
- At GOSH specimens are delivered to the laboratories by porters, and more urgent specimens via the pneumatic tube delivery system.

- Specimens sent by post outside GOSH must be packed and sent by microbiology as they need to be sent according to UN3373 regulations (United Nations Economic Commission for Europe, 2012). The specimen must be wrapped in a plastic bag, encased in an absorbent material within another plastic bag, placed in an approved plastic container within an approved cardboard box with the appropriate addressed infectious diseases stickers. A warning 'Pathological Sample' along with the sender's name and address must be visible on the outside. Specimens should not be sent to a laboratory outside the Trust direct from a ward, but sent through the microbiology laboratory to ensure correct packing and audit trail.
- In children suspected of suffering from viral haemorrhagic fevers such as Lassa fever, Marburg or Ebola virus, the Infection Control Team must be consulted before any specimens are taken (Advisory Committee on Dangerous Pathogens 2004; Advisory Committee on Dangerous Pathogens 1998; Advisory Committee on Dangerous Pathogens 1996). GOSH has no containment Level 4 facilities in the laboratory.
- Spillage kits (eg Clinell®) must be available for decontamination of any spillages of blood or body fluids. The manufacturer's instructions for use must be followed (Health and Safety Executive, 2014).

**Equipment**

This will vary according to the specimen required but must include:

- disposable gloves
- additional personal protective equipment (apron/gown, mask/respirator, visor - where applicable)
- a protective tray
- a sterile container for the specimen
- appropriate transport medium, if required
- laboratory specimen form
- a polythene transportation bag
- biohazard label, if required

**Specimen collection**

**Blood samples**

Blood sampling should be performed by a healthcare worker trained and competent in the procedure. As there are many haematological, biochemical, immunological and microbiological blood tests the person should seek information as to the appropriate laboratory containers required for specific tests and the amount of blood required.

This information is available on PiMS or in the individual departmental user manuals. Protective clothing such as gloves and aprons (and facial protection when appropriate) must be used along with the aseptic non-touch technique.

The 'Broken Needle Technique' (breaking the hub of the needle to obtain blood from small infants) poses an additional risk of injury to the child and user and must NOT be used.

**Blood culture**

Detection of microorganisms by culture of blood is essential in the diagnosis of bloodstream infections, including infective endocarditis, infections presenting as pyrexia of unknown origin, prosthetic material infections and intravenous catheter infections. Blood culture may also detect bacteraemia associated with primary infections such as pneumonia and septic arthritis. Accurate positive results provide valuable information to guide optimal antibiotic therapy early on which can improve outcome from these conditions.

On the other hand, contaminated blood cultures can cause considerable diagnostic confusion and lead to unnecessary or sub-optimal antimicrobial therapy.

Contamination may be prevented by careful collection of the blood using the aseptic non-touch-technique. The specimen should also preferably be taken during pyrexial episodes as more bacteria may be present at that time.

Blood cultures should be taken when there is a clinical need to do so in response to any of the following clinical signs suggestive of sepsis and a deteriorating clinical picture including:

- abnormalities in
    1. heart rate
    2. core temperature
    3. leucocyte count
- presence of rigors or chills
- Other focal signs of infection, such as pneumonia, septic arthritis, meningism, urinary tract infection including pyelonephritis and acute abdominal pathology.

Procedure (Department of Health (DH) 2007):

- Use both blood culture bottles (aerobic and anaerobic) and scrub the bung with a 2% chlorhexidine/70% alcohol wipe (eg Clinell®) for 15 seconds and allow to dry prior to inoculation.
- Prior to venepuncture soap and water should be used to clean any visibly soiled skin. The skin must then be decontaminated with a 2% chlorhexidine/70% alcohol applicator (eg Chloraprep®Sepp 0.67 ml) and allowed to dry. Do not re-palpate vein (even with gloved hand) after decontamination.
- After withdrawing the blood, insert the blood into the container with a new sterile needle. There is a risk of contamination of skin organisms on the needle used to withdraw the blood.
- When inoculating the blood culture bottles, the **anaerobic culture bottle should be inoculated first** and then the aerobic culture bottle, so that oxygen trapped in the syringe will not be transferred to the anaerobic bottle.
- Volume of blood is the most critical factor in the detection of blood stream infections. Place up to 4ml in the aerobic bottle (priority) and up to 10 ml in the anaerobic bottle, but ensure that when using both bottles, the anaerobic bottle is inoculated first. For neonates 1-2 mls of blood is recommended. However the sensitivity of neonatal blood cultures is increased if more blood is cultured.
- Inoculation of the blood into the blood culture bottles should be performed first before inserting blood into other bottles as many of these bottles are not sterile and accidental contamination may occur.
- Children with suspected central venous line sepsis, blood for culture may be taken from a peripheral vein stab and also from the appropriate intravascular lines to enable identification of colonisation of the line. In cases of suspected bacterial endocarditis three blood cultures should be taken from separate venepunctures to optimise recovery of bacteria which may be present low in numbers.
- Blood sampling for culture from a peripheral cannula: cultures from should only be taken from newly inserted peripheral cannulae if there is no alternative to obtain a blood sample for culture through a separate venepuncture. Strict asepsis must be maintained. The specimen must be clearly labelled indicating that the blood sample was taken from a peripheral cannula, as the risk of contamination is high.
- A poster with detailed illustration of the blood culture technique is available to GOSH staff internally on the GOSHweb intranet site.

**Analysis of antibiotic levels**

The relationship between drug dose, drug concentration in biological fluid and the individual child's metabolic process must be understood for interpreting results. The results may be affected by the route of administration, age of the child and disease process such as liver and renal disease. The analysis involves testing levels in blood serum or plasma in direct relationship to drug administration.

Routine in house drug level assay is available for amikacin, gentamicin, tobramycin and vancomycin. Other levels are referred and should be discussed.

- Individual antibiotic policies are on the Medicine and Pharmacy intranet web page (available to GOSH staff internally on the GOSHweb intranet site) and available upon request.
- Record on the laboratory form/sample the drug, dose and mode of administration, the time the drug is given and whether the sample is a 'peak' or 'trough' level.
- Trough levels are taken immediately prior to the time a drug is due; peak levels are taken one hour after bolus or infusion finished.
- Blood for antibiotic assay must not be taken through the same catheter which has been used to give the antibiotic at any time. Antibiotics bind to plastic and the drug may release intermittently giving false results.
- Spurious low or high level results may occur if blood is drawn from any central venous catheter and ideally levels should not be drawn from a central venous catheter.

**Biopsy material**

Specimens such as skin, muscle, kidney, liver, jejunal, tissue or brain biopsies are generally obtained by medical staff either under general or local anaesthetic according to the site. A sterile technique is required for all these procedures. All biopsy specimens must be discussed with the relevant laboratory personnel in order that:

- The most appropriate specimen and laboratory tests are undertaken. If the specimen is small it may be necessary to limit the range of tests.
- Check if the specimen is to be fixed in formalin. DO NOT use formalin if the specimen is for microbiological investigation. In many cases both histopathological and microbiological/virological analysis will be required and it is critical that separate specimens be sent for these purposes so they are processed and transported appropriately.

**Cerebrospinal fluid**

Sampling of cerebrospinal fluid is essential for the accurate diagnosis of infective meningitis and may aid in the diagnosis of encephalitis.

Cerebro-spinal fluid (CSF) is most commonly obtained via a lumbar puncture performed by medical staff. A sterile technique is required as there is a risk of introducing infection itself causing meningitis. Specimens of CSF should be dispatched to the laboratory immediately.

Outside normal working hours it is essential that the on-call laboratory staff are contacted when the sample is being transported. It is important not to store the specimen in a refrigerator as this may cause the cells to deteriorate or lyse giving rise to misleading results.

It is common practice to send three separate collection tubes of CSF when investigating for evidence of sub-arachnoid haemorrhage, as the initial part of the sample may be contaminated with blood from outside the sub-arachnoid space.

If this is performed, it is important to label the tubes as such and specifically request counts on the first and third samples. It is also important to remember that a CSF glucose level (sent separately to chemical pathology) can only be accurately interpreted in conjunction with a simultaneous plasma glucose level.

**Ear swabs**

No antibiotics or other therapeutic agents should have been in the aural region for about three hours prior to sampling the area as this may inhibit the growth of organisms.

- If there is purulent discharge this should be sampled.
- Place a sterile swab into the outer ear and gently rotate to collect the secretions.
- Place swab in transport medium.
- For deeper ear swabbing a speculum may be used. Experienced medical staff only should undertake this procedure as damage to the eardrum may occur.

**Eye swabs**

- Where possible ask the child to look upwards and gently pull the lower lid down or gently part the eyelids.
- Use a sterile cotton wool swab and gently role the swab over the conjunctival sac inside the lower lid. Hold the swab parallel to the cornea to avoid injury if the child moves.
- Place the swab in the transport medium.
- For suspected *Chlamydia trachomatis* infection obtain a Chlamydia sampling swab from the virology department. These tests are referred to an external laboratory and are infrequently performed, therefore swabs may not be immediately available and testing may be delayed pending request for swabs.
- Clean the eye first with sterile normal saline to obtain a clear view of the conjunctiva.
- Using the swab, part the eyelids and gently rub the conjunctival sac of the lower lid to obtain epithelial cells.
- Place the swab in the transport medium provided.
- Identification will be by the current molecular test employed at the referral laboratory.

**Stool samples**

- Please specify whether the sample is a routine (admission) screening sample or an investigation for suspected intestinal infection.
- If viral gastroenteritis (eg norovirus, rotavirus) is suspected, the stool specimen should be sent to the virology laboratory. To exclude a bacterial cause a second stool specimen can be sent to the microbiology laboratory.
- A faecal specimen is more suitable than a rectal swab.
- A specimen can be obtained from a nappy or clean potty/bedpan.
- Use the scoop attached to the inside of the lid of the specimen container to place faecal material into the container.
- Where diarrhoea is present, a small piece of non-absorbent material lining the nappy can be used to prevent material soaking into the nappy.
- Examine the sample for consistency, odour or blood and record observations to monitor changes.
- If segments of tapeworm are seen, send to the laboratory. Tapeworm segments can vary from the size of rice grains to a ribbon shape, one inch long.
- For the identification of *Enterobius vermicularis* (threadworm/pin worm) material should be obtained first thing in the morning on awakening by using a clear adhesive tape (eg Sellotape®) slide. Place the sticky side of a strip of tape over the anal region to obtain the material and stick the tape smoothly onto a glass slide. The worm can then be identified under the microscope. Thread worm lay their ova on the perianal skin at night and therefore will not be seen in a faecal specimen.
- Where acute amoebic dysentery is suspected, the specimen of stool must be freshly dispatched to the laboratory. The parasite causing amoebic dysentery exists in a free-living motile form and in

the form of non-motile cysts. Both forms are characteristic in their fresh state but the motile form cannot be identified when dead. 'Hot faeces' should be discussed with the laboratory prior to collection.

**Fungal samples of hair, nail and skin**

Special containers (Dermapak®) may be obtained from the microbiology department:

- Samples of infected hair should be removed by plucking the hair with forceps or gloves. The root of the hair is infected not the shaft.
- Samples of the whole thickness of the nail or deep scrapings should be obtained.
- The skin should be cleaned with an alcohol swab. Epidermal scales scraped from the active edge of a lesion or the roof of any vesicle should be obtained.

**Sputum**

Good quality sputum samples are essential for accurate microbiological diagnosis of pneumonia but also acute tracheitis and bronchitis. Samples contaminated with oro-pharyngeal secretions and saliva are difficult to interpret and can be misleading.

- Encourage the child to cough especially after sleep and expectorate into a container. Alternatively, a poorer sample may be obtained as from naso-pharyngeal/tracheal suction using a sputum trap can be undertaken.
- Physiotherapy may help to facilitate expectoration.
- Ensure the material obtained is sputum and not saliva

**Throat swabs**

- Specify on the laboratory form if this is a routine admission screen for MRSA or a swab for the investigation of a suspected infection.
- For the collection of throat swabs as part of screening for MRSA refer to the policy for the control of Methicillin-resistant Staphylococcus aureus (MRSA).
- Place the child in a position with a good light source. This will ensure maximum visibility of the tonsillar bed.
- Either depress the tongue with a spatula or ask the child to say "aahh". The procedure is likely to cause gagging and the tongue will move to the roof of the mouth. This can prevent accurate sampling, therefore it is important to quickly but gently rub the swab over the ansillar fossa (tonsillar bed) or area where there is exudate or a lesion.
- Care should be taken not to contaminate the swab by contact with the tongue or the oral mucosa on removal.
- Return swab to the container with transport medium.

***Procedure for throat swab***

- Place the child in a position with a good light source. This will ensure maximum visibility of the tonsillar bed.
- Either depress the tongue with a spatula or ask the child to say "aahh". The procedure is likely to cause gagging and the tongue will move to the roof of the mouth. This can prevent accurate sampling, therefore it is important to quickly but gently rub the swab over the ansillar fossa (tonsillar bed).
- Care should be taken not to contaminate the swab by contact with the tongue or the oral mucosa on removal.
- Return swab to the container with transport medium.

Patients that have previously been screened positive for MRSA should in addition to the nose and throat swabs also have swabs taken from the following sites:

- hairline (swab along outline of scalp hair)

- axillae
- groin
- perineum
- any wounds or skin lesions
- umbilicus (only in neonates)
- insertion sites of devices (eg tracheostomy, gastrostomy, central venous catheter)

When taking samples from intact skin the swab should be moistened with sterile 0.9% saline solution before sampling as this assists in the transfer of bacteria from the sampling site to swab and can increase the number of micro-organisms collected.

**Urine**

Bedside urine testing for the presence of blood, protein and other analytes is usually undertaken with reagent strips the results of which indicate that further laboratory investigation is required.

Most urine samples sent to the microbiology laboratory are for bacteriological investigation. The same collection techniques also apply to samples sent for virological investigation.

Urine samples should be dispatched to the laboratory as soon as possible or no more than four hours if kept at room temperature or up to 24 hours if kept at four degrees centigrade to avoid overgrowth of organisms and misleading results.

All methods used to collect urine samples can result in contamination with bacteria from outside the bladder. This can lead to an inaccurate diagnosis, involve unnecessary treatment or require a sample to be repeated which has implications for patient care and cost effectiveness.

Normal social hygiene, such as washing the genitalia with soap and water and drying thoroughly is considered sufficient to minimise contamination from the skin prior to collection of the specimen. Assess the clinical and psychosocial needs of the child as to whether cleaning the genitalia is necessary. The nurse must be sensitive to the cultural issues surrounding touching intimate parts of the body.

It can be difficult to obtain a urine sample from incontinent or non-toilet trained children and the most popular non-invasive method is the midstream or ‘clean catch’ specimen.

***Midstream or 'clean catch' specimen***

The 'clean catch' method is the most popular non-invasive method.

- Ensure that the child’s/young person’s genitalia have been washed with soap and water and dried thoroughly. Ask the child/young person to wash hands with soap and water.
- In the female encourage separation of the labia whilst passing urine.
- In the male encourage retraction of the prepuce, if appropriate, whilst passing urine.
- Ask the child/young person to void a small amount of urine into the toilet first.
- Then ask the child/young person to urinate 10-20 ml directly into the specimen container.
- Instruct the child/young person that the remaining urine can passed into the toilet.
- Place the lid securely on the specimen container. Wipe the outside of the container with a sanitising wipe and place container in polythene transport bag.

***Pad or bag specimen***

Urine collection pads or urine collection bags are often used for incontinent or non toilet trained children, but are more susceptible to contamination due to close contact with the anogenital area. Urine collection pads as the next best option to clean catch. When using urine collection pads, manufacturer’s instructions should be followed. Cotton-wool balls, gauze- or sanitary pads should not be used.

Urine collection pads:

- Remove nappy and clean perineum or prepuce of the infant with soap and water. Do not apply any creams.
- Place the urine collection pad across vulva or penis in a lengthwise fashion.

- Remove the adhesive backing from the pad and secure to nappy.
- Change urine collection pad every 30-45 minutes and also when the child has passed stool, to reduce the risk of contamination with skin-or faecal flora.
- Once the child has passed urine remove the nappy with the urine collection pad in it.
- Lay the pad down wet side up on an appropriate clean surface.
- Take sterile 5 ml syringe and place the tip on the pad. Extract the urine by pulling up the plunger.
- Repeat until required amount of urine is obtained.
- Empty syringe into a sterile container.

Urine collection bags:

- Select the correct size sterile urine bag to avoid leakage or contamination with faeces.
- Remove nappy and clean the perineum or prepuce of the infant with soap and water.
- Dry area thoroughly and do not apply any creams.
- Remove the protective backing from the bag, then
  - For the female, place the bag over the vulva, starting from the perineum and working upwards, pressing the adhesive to perineum and symphysis.
  - For the male, insert penis and scrotum into the opening of the bag and press adhesive to perineum and symphysis.
- Cut a hole in the diaper and pull the urine bag through the opening.
- Once the child has passed urine, perform hand hygiene, put on gloves and remove the bag.
- Hold the bag over a sterile urine specimen container and cut off the tip of the bottom corner of the bag. Empty the urine into the container.
- Wash the genitalia after the procedure to prevent soreness of the skin.

***Suprapubic aspirate***

Collection of urine by a supra-pubic aspirate should be considered when a sterile sample is required. Ultrasound guidance should be used to indicate the presence of urine in the bladder before a suprapubic aspirate is attempted.

***Catheter specimen***

This is collected from the self-sealing bung of the urinary drainage tubing in a child who is already catheterised. Do not disconnect the closed drainage system as infection may be introduced nor take the sample from the urinary drainage bag as the specimen maybe contaminated.

- Using an aseptic non-touch technique, clean the catheter sampling site with 2% chlorhexidine/70% alcohol wipe (eg Clinell®) and allow to dry.
- Collect the urine using appropriate sterile equipment appropriate to access port ie either using a sterile syringe and needle and inserting the needle into the bung at an angle of 45 degrees; this will minimise penetration of the wall of the tubing and subsequent needle stick injury, or a needle-less system.
- Gently withdraw the urine into the syringe.
- Remove the needle and syringe, wipe the area with the alcohol swab and allow to dry. The rubber bung will self-seal.
- Place the urine in a sterile container.
- Discard the needle and syringe into a sharps container.

***Obtaining urine from a Mitroffanof stoma***

The specimen should be obtained by a nurse who is familiar with the Mitroffanof operation and the specific anatomy of the area on the child.

- The specimen should ideally be taken in conjunction with normal bladder emptying.
- A new sterile catheter of the child's normal catheter size should be used.
- Clean the stoma with soap and water and dry.
- Gently insert the lubricated sterile catheter into the stoma and collect the urine into a sterile container. A water-soluble lubricant should be used.
- Ensure the bladder is completely empty before withdrawing the catheter.
- Wipe the area dry with a tissue.

**Wound swabs**

Interpretation of results must be in conjunction with clinical signs. In the absence of clinical signs of infection wound swabs will provide little if any useful information and simply reflects colonisation.

- Obtain the specimen prior to any dressing or cleaning procedure of the wound. This will maximise the material obtained and prevent killing of the organism by the use of antiseptics.
- Use a sterile swab and gently rotate on the area to collect exudate from the wound and place into transport medium. Where there is pus collect as much as possible in a sterile syringe or sterile container (do not use a swab) and send to the laboratory.
- For detection of *Mycobacterium tuberculosis*, pus collected neat in to a pot or tissue biopsy is preferred, however a calcium alginate swab can be used. The alginate swab gradually dissolves maximising the isolation of the organism as the numbers of organisms are usually small.

**ANTIBIOTIC SUSCEPTIBILITY TESTING**

Antibiotic sensitivity is the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method

.**ANTIBIOTICSENSITIVITY**

Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth. Other methods to test antimicrobial susceptibility include the Stokes method, E-test (also based on antibiotic diffusion) agar and Broth dilution methods for Minimum Inhibitory Concentration determination.

Ideal antibiotic therapy is based on determination of the etiological agent and its relevant antibiotic sensitivity. Empiric treatment is often started before laboratory microbiological reports are available when treatment should not be delayed due to the seriousness of the disease. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to resist or inactivate the antibiotic. Some antibiotics actually kill the bacteria (bactericidal), whereas others merely prevent the bacteria from multiplying (bacteriostatic) so that the host's immune system can overcome them. Müeller-Hinton agar is most frequently used in this antibiotic susceptibility test.

**Mueller-Hinton agar**

MH agar is considered the best medium to use for routine susceptibility testing of nonfastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing
- It is low in sulfonamide, trimethoprim, and tetracycline inhibitors
- It supports satisfactory growth of most nonfastidious pathogens

A large body of data and experience has been collected concerning susceptibility tests performed with this medium. Please note that the use of media other than Mueller-Hinton agar may result in erroneous

results. Also note that only the aerobic or facultative bacteria that grow well on unsupplemented MH agar should be tested using this protocol. Fastidious organisms require MH agar supplemented with additional nutrients and require that modification to this protocol be made. Neither the supplements nor the procedural modification are discussed in this basic protocol.

Plates that are too shallow will produce false susceptible results as the antimicrobial compound will diffuse further than it should, creating larger zones of inhibition. Conversely, plates poured to a depth >4 mm will result in false resistant results.

pH of the MH agar should fall between 7.2 and 7.4 at room temperature after solidification and should be tested when the media is first prepared. If the pH is <7.2 certain drugs will appear to lose potency (aminoglycosides, quinolones, macrolides), while other agents may appear to have excessive activity (tetracycline). If the pH is >7.4, the opposite results may occur.

Excessive thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim resulting in smaller and less distinct zones of inhibition, or no zones at all.

The incorrect concentration of divalent cations (calcium and magnesium) will affect the results of aminoglycoside and tetracycline tests against *Pseudomonas aeruginosa*. Excess cation concentration will result in reduced zone sizes and low concentration will increase zone sizes. Excess calcium will increase the zone size of *P. aeruginosa* against daptomycin. Excess zinc ions may reduce the zone size of carbapenems against *P. aeruginosa.*

**McFarland Standard**

McFarland standards are suspensions of either barium sulfate or latex particles that allow visual comparison of bacterial density commercially prepared standards are available for purchase from companies such as Remel or BD BBL. These often include a Wickerham card, which is a small card containing parallel black lines. A 0.5 McFarland standard is equivalent to a bacterial suspension containing between 1 x 108 and 2 x 108 CFU/ml of E. coli. A 0.5 McFarland standard may be prepared in-house as describe below.

- Add a 0.5-ml aliquot of a 0.048 mol/liter BaCl2 (1.175% wt/vol BaCl2 • 2H20) to 99.5 ml of 0.18 mol/liter H2SO4 (1% vol/vol) with constant stirring to maintain a suspension.
- Verify the correct density of the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvette. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standard.
- Transfer the barium sulfate suspension in 4- to 6-ml aliquots into screw-cap tubes of the same size as those used in standardizing the bacterial inoculums.
- Tightly seal the tubes and store in the dark at room temperature.

**Possible Questions**

**Part B (Two marks)**

1. Define Medical Microbiology.
2. Define Medical Bacteriology.
3. What are specimens..
4. Explain the precaution to undergo in collection of specimens.
5. Define transport media.
6. Define antibiotics
7. Define disinfectant
8. Define midstream urine
9. Outline the lancet prick method of blood collection.
10. Name any two transport media.

**Part C (Eight marks)**

1. Give a detailed note on laboratory precaution.
2. Discuss about the personal safety in laboratory.
3. Write a detailed note on environmental safety to be observed in biological specimen disposal?
4. Explain about the antibiotic sensitivity testing.
5. Explain in detail about disc diffusion method.
6. Give the protocol and importance of well diffusion method.
7. Describe the role of antibiotics.
8. How will you examine the pathological specimen?
9. What are the methods of isolation of pathogenic organism?
10. Why it is necessary to collect specimen?
11. Give the outline of biochemical examination of specimen.
12. Explain processing of pathological specimen.
11. Discuss about collection of sputum.
12. Comment on urine collection.
13. Comment on stool specimen collection.
13. Write about the vein puncture method of blood collection?

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS: I M.Sc MB** **COURSE NAME: MEDICAL BACTERIOLOGY**

**COURSE CODE: 19MBP202** **UNIT: I** **BATCH-2019-2021**

| S.No | Unit I Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | The sputum specimen must be stored at | 10C | 5C | 4C | 8C | 4C |
| 2 | the blood samples should be collected at the stage of the disease. | late | early | before diagnosis | after symptom | early |
| 3 | When a parasite is growing and multiplying within a host, it is said have | pathogen | infection | organism | diagnose | infection |
| 4 | For microbiological examination urine must be collected as a specimen | good | sterile | normal | clean catch mid-stream | clean catch mid-stream |
| 5 | Cary-Blair medium is used for the transportation of specimen. | blood | CSF | stool | urine | stool |
| 6 | a parasite organism or agent that produce such a disease is a | pathagon | parasite | bacteria | virus | pathagon |
| 7 | the ability of an organism to cause disease is called | diagnosis | symptoms | pathogenicity | disease | pathogenicity |
| 8 | The most important step in the diagnosis of an infection is the proper collection of _____. | Specimen | Organism | Symptoms | Prophylaxis | Specimen |
| 9 | In case of meningeal irritation or affected cerebrum specimen is collected. | blood | cerebrospinal fluid | urine | pus | cerebrospinal fluid |
| 10 | in blood specimens is seperated and used for serological techniques. | haemoglobin | serum | iron | protein | serum |
| 11 | The collected CSF should be stored at | room temperature | 4C | 2C | 5C | room temperature |
| 12 | is a good transport medium for stool sample. | agar medium | peptone water | Cary-Blair medium | alkaline medium | Cary-Blair medium |
| 13 | An alternate transport medium for stool sample is | alkaline-peptone water | peptone water | alkaline medium | water | alkaline-peptone water |
| 14 | A sterile is used for transportation of specimens to labs. | container | plastic container | screw-cap container | metal container | screw-cap container |
| 15 | Specimens must be collected before administration of _____ | Vaccines | Antimicrobial drugs | Symptoms | Culturing | Antimicrobial drugs |
| 16 | To eliminate normal flora skin surface is cleansed with _____. | Germicide | Soap | Vaseline | Cotton | Germicide |
| 17 | Pus swabs collected in transport medium should reach lab within ____ hours | 2 | 4 | 6 | 8 | 6 |
| 18 | During blood collection the skin should be vein punctured by | Isopropyl | Antiseptic | Soap | Antibiotic | Isopropyl |

| | | | | | | |
|---|---|---|---|---|---|---|
| | cleansing with _____. | alcohol | | | | alcohol |
| 19 | About _____ ml of blood should be collected. | 10 | 20 | 30 | 40 | 20 |
| 20 | the blood samples should be incubated at _____. | 25C | 37C | 4C | 10C | 37C |
| 21 | _____ ml of urine sample should be collected. | 10 | 40 | 30 | 20 | 20 |
| 22 | In case of _____ only few millilitres of urine specimen can be collected. | Arthritis | Gas gangrene | Renal failure | Paralysis | Renal failure |
| 23 | If the urine sample is delayed for more than an hour _____ can be added. | Amino acid | Boric acid | Sodium chlloride | Calcium chloride | Boric acid |
| 24 | In case of suspected renal tuberculosis the urine should be collected for _____ successive mornings | 1 | 2 | 3 | 4 | 3 |
| 25 | The CSF should be collected from _____ | Cerebrum | Cerebellum | Ventricle | Arachnoid space | Arachnoid space |
| 26 | In case of infants the CSF should be collected by puncturing _____. | Ventricle | Arachnoid space | Cerebrum | Cerebellum | Ventricle |
| 27 | About _____ ml of CSF should be collected in sterile tubes. | 10 | 30 | 40 | 20 | 20 |
| 28 | For collecting nasopharyngeal aspirates _____ is passed gently through one nostril. | Sterile catheter | Syringe | Swab | Cotton | Sterile catheter |
| 29 | _____ is collected in case of eye specimens. | Pus | Mucous | Conjuctival scrapings | Conjunctival tissue | Conjuctival scrapings |
| 30 | Respiratory secretions should be transported to laboratory within _____.hours | 1 | 2 | 3 | 4 | 2 |
| 31 | The natural secretions of eye contains _____ enzymes | Antibiotic | Toxins | Antibacterial | Amylase | Antibacterial |
| 32 | If not possible to obtain faeces a specimen is collected by inserting _____ | Cotton woll swab | Syringe | Catheter | Cotton | Cotton woll swab |
| 33 | Salmonella, Shigella and Vibrio survive in Cary Blair medium for _____ hrs. | 24 | 48 | 32 | 76 | 48 |
| 34 | Campylobacter can survive in C-B medium for ___hes. | 2 | 4 | 6 | 8 | 6 |
| 35 | If cholera is suspected the stool sample should be sent to the lab within _____- | 1 | 3 | 5 | 8 | 8 |
| 36 | _____ is the transportation medium used in case of cholera. | CB medium | Boric acid | Alkaline peptone water | Phosphate buffered saline | Alkaline peptone water |
| 37 | For suspected viral enteritis the stool sample is transferred to | Phosphate | Alkaline | CB medium | Boric acid | Phosphate |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 9ml of _____ | buffered saline | peptone water | | | buffered saline |
| 38 | About _____ ml of sterile phosphate buffered saline is needed for suspected viral enteritis | 3 | 6 | 9 | 12 | 9 |
| 39 | _____ is the transportation medium used for the collection of synovial, pleural or asceptic fluid samples | Trisodium citrate | Sodium chlloride | Boric acid | Calcium chloride | Trisodium citrate |
| 40 | About _____ ml of synovial, pleural and ascitic samples were collected. | 2 to 3 | 5 to 6 | 3 to 5 | 4 to 6 | 2 to 3 |
| 41 | Synovial, pleural and ascitic fluids should be mixed with _____. | Antibiotic | Antiseptic | Anticoagulant | Antibacterial | Anticoagulant |
| 42 | In case of sputum sample the sample should be collected best in _____. | morning | Evening | Mid night | Noon | morning |
| 43 | _____ bacilli requires entirely different method of identification | Rod shaped | Spindle shaped | Club shaped | Acid-fast | Acid-fast |
| 44 | In suspected anthrax the pus sample should be collected from _____. | Lesions | Scrapings | Edema | Necrosis | Lesions |
| 45 | For darkfield examination pus from syphilitic ulcers are suspected in 0.2ml of _____. | Salt solution | Saline solution | Anticoagulant | Antiseptic | Saline solution |
| 46 | In case of tuberculosis _____ specimen is collected | Stool | Urine | Blood | Sputum | Sputum |
| 47 | The sputum sample collected in paper towel should be soaked in _____-. | Saline solution | Anticoagulant | Formalin solution | Salt solution | Formalin solution |
| 48 | _____ technique is used to stain acid fast bacilli | Ziehl Neelsen | Gram staining | Endospore staining | Quellung | Ziehl Neelsen |
| 49 | In bacilliary dysentry _____ yields more positive cultures than stools. | Smears | Swabs | Rectal swabs | Renal smears | Rectal swabs |
| 50 | In case of salmonellosis _____ sample is collected. | Urine | Stool | Sputum | Blood | Blood |
| 51 | In case of thyphoid _____ sample should be collected. | Sputum | Blood | Faeces | CSF | Blood |
| 52 | In order to avoid drying of stool sample the swabs should be placed in _____. | Thioglycollate semisolid medium | Sodium citrate | Buffered glycerol water | Para amino benzoic acid | Thioglycollate semisolid medium |
| 53 | A n alternative for Thioglycollate semisolid medium is _____. | Sodium citrate | Buffered glycerol water | Para amino benzoic acid | Thioglycollate semisolid medium | Buffered glycerol water |

| | | | | | | |
|---|---|---|---|---|---|---|
| 54 | _____ should be incorporated in all blood culture media | Trisodium citrate | Formalin solution | Sodium citrate | Para amino benzoic acid | Para amino benzoic acid |
| 55 | _____ is added to the blood sample to prevent coagulation | Sodium citrate | Thioglycollate semisolid medium | Antiseptic | Antibacterial | Sodium citrate |
| 56 | Once the specimen is collected the container should be _____. | Labelled | Opened | Diluted | Defined | Labelled |
| 57 | Samples of blood and CSF for culture should not be _____ | Labelled | Refrigerated | Processed | Incubated | Refrigerated |
| 58 | The sputum sample should be free from _____ | Blood | Pus | Saliva | Tissue | Saliva |
| 59 | when the patient has symptoms of cystitis _____ should be collected. | blood | Pus | Sputum | urine | urine |
| 60 | In case of suspected septicemia _____ sample should be collected | blood | Pus | Sputum | urine | blood |

## UNIT-II
## SYLLABUS

Infections – types – methods – infectious disease cycle. Definitions of Epidemics, Endemics Pandemics and investigation of epidemics and control. Definition of pathogens, Saprophytes and Commensals. Quality control in microbiology lab.

### Infection

**Infection** is the invasion of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to these organisms and the toxins they produce. **Infectious** disease, also known as transmissible disease or communicable disease, is illness resulting from an **infection**.

A primary infection is the first time you are exposed to and infected by a pathogen. During a primary infection, your body has no innate defenses against the organism, such as antibodies.

A secondary infection can occur when a different infection, known as the primary infection, makes a person more susceptible to disease. It is called a secondary infection because it occurs either after or because of another infection.

A second infection that follows recovery from aprevious infection by the same causative agent.

Iatrogenic infections are those infections transmitted during medical treatment and care.

A hospital-acquired infection (HAI), also known as a nosocomial infection, is an infection that is acquired in a hospital or other health care facility.

### The Infectious Disease Cycle

The **infectious disease cycle** or **chain of infection** represents these events in the form of an intriguing epidemiological mystery story


### What Pathogen Caused the Disease?

The first link in the infectious disease cycle is the pathogen. After an infectious disease has been recognized in a population, epidemiologists must correlate the disease outbreak with a specific pathogen. The disease's exact cause must be discovered. This is where Koch's postulates, and modifications of them, are used to determine the etiology or cause of an infectious disease. At this point the clinical or diagnostic microbiology laboratory enters the investigation. Its purpose is to isolate and identify the pathogen that caused the disease and to determine the pathogen's susceptibility to antimicrobial agents or methods that may assist in its eradication.

Many pathogens can cause infectious diseases in humans and will be discussed in detail in chapters 38 to 40. Often these pathogens are transmissible from one individual to another. A **communicable disease** is an illness caused by a pathogen or its products that has been transmitted from an infected person or a reservoir, either directly or indirectly. Pathogens have the potential to produce disease (pathogenicity); this potential is a function of such factors as the number of pathogens, their virulence, and the nature and magnitude of host defenses.

### What Was the Source and/or Reservoir of the Pathogen?

The source and/or reservoir of a pathogen is the second link in the infectious disease cycle. Identifying the source and/or reservoir is an important aspect of epidemiology. If the source or

reservoir of the infection can be eliminated or controlled, the infectious disease cycle itself will be interrupted and transmission of the
pathogen will be prevented.

A **source** is the location from which the pathogen is immediately transmitted to the host, either directly through the environment or indirectly through an intermediate agent. The source can be either animate (e.g., humans or animals) or inanimate (e.g., water, soil, or food). The **period of infectivity** is the time during which the source is infectious or is disseminating the pathogen.

The **reservoir** is the site or natural environmental location in which the pathogen is normally found living and from which infection of the host can occur. Thus a reservoir sometimes functions as a source. Reservoirs also can be animate or inanimate.

Much of the time, human hosts are the most important animate sources of the pathogen and are called carriers. A **carrier** is an infected individual who is a potential source of infection for others. Carriers play an important role in the epidemiology of disease.

Four types of carriers are recognized:

1. An **active carrier** is an individual who has an overt clinical case of the disease.
2. A **convalescent carrier** is an individual who has recovered from the infectious disease but continues to harbor large numbers of the pathogen.
3. A **healthy carrier** is an individual who harbors the pathogen but is not ill.
4. An **incubatory carrier** is an individual who is incubating the pathogen in large numbers but is not yet ill.

Convalescent, healthy, and incubatory carriers may harbor the pathogen for only a brief period (hours, days, or weeks) and then are called **casual, acute,** or **transient carriers.** If they harbor the pathogen for long periods (months, years, or life), they are called **chronic carriers.**

As noted earlier, infectious diseases called zoonoses occur in animals and are occasionally transmitted to humans; thus these animals also can serve as reservoirs. Humans contract the pathogen by several mechanisms: coming into direct contact with diseased animal flesh (tularemia); drinking contaminated cow's milk (tuberculosis and brucellosis); inhaling dust particles contaminated by animal excreta or products (Q fever, anthrax); or eating insufficiently cooked infected flesh (anthrax, trichinosis). In addition, being bitten by arthropod **vectors** (organisms that spread disease from one host to another) such as mosquitoes, ticks, fleas, mites, or biting flies (equine encephalomyelitis and malaria, Lyme disease, Rocky Mountain spotted fever, plague, scrub typhus, and tularemia); or being bitten by a diseased animal (rabies) can lead to infection.

**How Was the Pathogen Transmitted?**

To maintain an active infectious disease in a human population, the pathogen must be transmitted from one host or source to another. Transmission is the third link in the infectious disease cycle and occurs by four main routes: airborne, contact, vehicle, and vector-borne.

**Airborne Transmission**

Because air is not a suitable medium for the growth of a pathogen, any pathogen that is airborne must have originated from a source such as humans, other animals, plants, soil, food, or water. In **airborne transmission** the pathogen is truly suspended in the air and travels over a meter or more from the source to the host. The
pathogen can be contained within droplet nuclei or dust.

**Droplet nuclei** are small particles, 1 to 4 _m in diameter, that result from the evaporation of larger particles (10 _m or more in diameter) called droplets. Droplet nuclei can remain airborne for hours or days and travel long distances. When animals or humans are the source of the

airborne pathogen, it usually is propelled from the respiratory tract into the air by an individual's coughing, sneezing, or vocalization. For example, enormous numbers of moisture droplets are aerosoled during a typical sneeze. Each droplet is about 10 _m
in diameter and initially moves about 100 m/second or more than 200 mi/hour!
Dust also is an important route of airborne transmission. At times a pathogen adheres to dust particles and contributes to the number of airborne pathogens when the dust is resuspended by some disturbance. A pathogen that can survive for relatively long periods in or on dust creates an epidemiological problem, particularly in hospitals, where dust can be the source of hospital acquired infections.

**Contact Transmission**

**Contact transmission** implies the coming together or touching of the source or reservoir of the pathogen and the host. Contact can be direct, indirect, or by droplet spread. Direct contact implies an actual physical interaction with the infectious source. This route is frequently called person-to-person contact. Person-to-person transmission occurs primarily by touching, kissing, or sexual contact (sexually transmitted diseases); by contact
with oral secretions or body lesions (herpes and boils); by nursing mothers (staphylococcal infections); and through the placenta (AIDS, syphilis). Some infectious pathogens also can be transmitted by direct contact with animals or animal products (*Salmonella* and *Campylobacter*).
Indirect contact refers to the transmission of the pathogen from the source to the host through an intermediary—most often an inanimate object. The intermediary is usually contaminated by an animate source. Common examples of intermediary inanimate objects include thermometers, eating utensils, drinking cups, andbedding. *Pseudomonas* bacteria are easily transmitted by this route. This mode of transmission is often also considered a form of vehicle transmission (see next section).
In droplet spread the pathogen is carried on particles larger than 5 _m. The route is through the air but only for a very short distance—usually less than a meter. Because these particles are large, they quickly settle out of the air. As a result droplet transmission of a pathogen depends on the proximity of the source and the host. Measles is an example of a droplet-spread disease.

**Vehicle Transmission**

Inanimate materials or objects involved in pathogen transmission are called **vehicles.** In **common vehicle transmission** a single inanimate vehicle or source serves to spread the pathogen to multiple hosts but does not support its reproduction. Examples include surgical instruments, bedding, and eating utensils. In epidemiology these common vehicles are called **fomites** [s., fomes or fomite]. A single source containing pathogens (blood, drugs, IV fluids) can contaminate a common vehicle that causes multiple infections. Food and water are important common vehicles for many human diseases.

**Vector-Borne Transmission**

As noted earlier, living transmitters of a pathogen are called vectors. Most vectors are arthropods (insects, ticks, mites, fleas) or vertebrates (dogs, cats, skunks, bats). **Vector-borne transmission** can be either external or internal. In external (mechanical) transmission the pathogen is carried on the body surface of a vector. Carriage is passive, with no growth of the pathogen during transmission. An example would be flies carrying *Shigella* organisms on their feet from a fecal source to a plate of food that a person is eating. In internal transmission the pathogen is carried within the vector. Here it can go into either a harborage or biologic transmission phase.
In **harborage transmission** the pathogen does not undergo morphological or physiological changes within the vector. An example would be the transmission of *Yersinia pestis* (the

etiologic agent of plague) by the rat flea from rat to human. **Biologic transmission** implies that the pathogen does go through a morphological or physiological change within the vector. An example would be the developmental sequence of the malarial parasite inside its mosquito vector.

**Why Was the Host Susceptible to the Pathogen?**

The fourth link in the infectious disease cycle is the host. The susceptibility of the host to a pathogen depends on both the pathogenicity of the organism and the nonspecific and specific defense mechanisms of the host.

**How Did the Pathogen Leave the Host?**

The fifth and last link in the infectious disease cycle is release or exit of the pathogen from the host. It is equally important that the pathogen escapes from its host as it is that the pathogen originally contacts and enters the host. Unless a successful escape occurs, the disease cycle will be interrupted and the pathogenic species will not be perpetuated. Escape can be active or passive, although often a combination of the two occurs. Active escape takes place when a pathogen actively moves to a portal of exit and leaves the host. Examples include the many parasitic helminths that migrate through the body of their host, eventually reaching the surface and exiting. Passive escape occurs when a pathogen or its progeny leaves the host in feces, urine, droplets, saliva, or desquamated cells. Microorganisms usually employ passive escape mechanisms.

**Endemic:** a disease that exists permanently in a particular region or population. Malaria is a constant worry in parts of Africa

**Epidemic:** An outbreak of disease that attacks many peoples at about the same time and may spread through one or several communities

**Pandemic:** When an epidemic spreads throughout the world

An **epidemic** is the slow spread of infectious disease to a large number of people in a given population within a short period of time, usually two weeks or less. For example, in meningococcal infections, an attack rate in excess of 15 cases per 100,000 people for two consecutive weeks is considered an epidemic.

Epidemics of infectious disease are generally caused by several factors including a change in the ecology of the host population (e.g. increased stress or increase in the density of a vector species), a genetic change in the pathogen reservoir or the introduction of an emerging pathogen to a host population (by movement of pathogen or host). Generally, an epidemic occurs when host immunity to either an established pathogen or newly emerging novel pathogen is suddenly reduced below that found in the endemic equilibrium and the transmission threshold is exceeded.

An epidemic may be restricted to one location; however, if it spreads to other countries or continents and affects a substantial number of people, it may be termed a pandemic.[1] The declaration of an epidemic usually requires a good understanding of a baseline rate of incidence; epidemics for certain diseases, such as influenza, are defined as reaching some defined increase in incidence above this baseline. A few cases of a very rare disease may be classified as an epidemic, while many cases of a common disease (such as the common cold) would not.

In epidemiology, an infection is said to be **endemic** (from Greek ἐν *en* "in, within" and δῆμος *demos* "people") in a population when that infection is maintained in the population without the need for external inputs. For example, chickenpox is endemic (steady state) in the UK, but malaria is not. Every year, there are a few cases of malaria reported in the UK, but these do not lead to sustained transmission in the population due to the lack of a suitable vector (mosquitoes of the genus *Anopheles*).

A **pandemic** (from Greek πᾶν *pan* "all" and δῆμος *demos* "people") is an epidemic of infectious disease that has spread through human populations across a large region; for instance multiple continents, or even worldwide. A widespread endemic disease that is stable in terms of how many people are getting sick from it is not a pandemic. Further, flu pandemics generally exclude recurrences of seasonal flu. Throughout history there have been a number of pandemics, such as smallpox and tuberculosis. One of the most devastating pandemics was the Black Death, killing over 75 million people in 1350. The most recent pandemics include the HIV pandemic as well as the1918 and 2009 H1N1 pandemics.

For an infection that relies on person-to-person transmission to be endemic, each person who becomes infected with the disease must pass it on to one other person on average. Assuming a completely susceptible population, that means that the basic reproduction number ($R_0$) of the infection must equal 1. In a population with some immune individuals, the basic reproduction number multiplied by the proportion of susceptible individuals in the population (*S*) must be 1. This takes account of the probability of each individual to whom the disease may be transmitted actually being susceptible to it, effectively discounting the immune sector of the population.

**Parasites** are plants or animals that live on or in a host getting their nutrients from that host. A **host**is an organism that supports a parasite. Sometimes the host is harmed by the parasite, and sometimes the relationship is neutral. But the host never benefits from the arrangement. When the parasite does have a negative impact on the host, it doesn't often kill the host directly, but the stressors that come with having parasites can kill.

**Ectoparasites** are parasites that live outside the body. In animals, they live on the skin and can cause itching and rashes. **Endoparasites** are parasites that live inside the body. For instance, they may live in the blood system, muscles, liver, brain, or digestive systems of animals.

Parasites are not one-size-fits-all. There are several main categories of parasites that can affect animals and plants. Probably the most-studied parasites are the ones that affect humans. Broadly grouped, they include protozoa, helminths, and arthropods. More familiar, less mysterious names are lice, ticks, mites, bed bugs, flukes, and tapeworms.

**Saprophytes** may refer to

- Saprotroph, a term used for organisms which obtain nutrients from dead organic matter (this term commonly applies to fungi)
- Saprophytes are more generally plants, fungi, or micro-organisms more accurately called myco-heterotrophs because they actually parasitize fungi, rather than dead organic matter directly. They live on dead or decomposing matter.

**commensal**

**1.** living on or within another organism, and deriving benefit without harming or benefiting the h ostindividual.

**2.** a parasitic organism that causes no harm to the host.

**Pathogen**

In biology, a **pathogen**(Greek: πάθος *pathos* "suffering, passion" and -γενής -*genēs* "producer of") in the oldest and broadest sense is anything that can produce disease, a term which came into use in the 1880s. Typically the term is used to describe an infectious agent such as a virus, bacterium, prion, fungus, viroid, or parasite that causes disease in its host. The host may be a human, an animal, a plant, a fungus, or even another micro-organism.

There are several substrates including *pathways* where the pathogens can invade a host. The principal pathways have different episodic time frames, but soil contamination has the longest or

most persistent potential for harboring a pathogen. Diseases caused by organisms in humans are known as pathogenic diseases.

**Pathogenicity** is the potential disease-causing capacity of pathogens. Pathogenicity is related to virulence in meaning, but some authorities have come to distinguish it as a *qualitative* term, whereas the latter is *quantitative*. By this standard, an organism may be said to be pathogenic or non-pathogenic in a particular context, but not "more pathogenic" than another. Such comparisons are described instead in terms of relative virulence. Pathogenicity is also distinct from the transmissibility of the virus, which quantifies the risk of infection.

A pathogen may be described in terms of its ability to produce toxins, enter tissue, colonize, hijack nutrients, and its ability to immune suppress the host.

## QUALITY CONTROL IN MICROBIOLOGY LAB

### Laboratory solution

- Integrated controls of fume hoods, monitoring devices and value-added services
- Demand controlled ventilation of the laboratory rooms
- Controls and conditioning of the supply air in the primary plant
- Operation and monitoring of the facilities on management level
- Fast and reliable fire detectors sensing smoke, heat, flames and CO at an early stage
- Tightly controlled differential pressure to protect people from toxic substances and preventing cross-contamination
- Safe evacuation independent of the cause with clear automated audio and visual commands and opening of escape routes
- Integrated and flexible access control systems preventing unauthorized entry, reduce risk of contamination or product tampering and combine several sources of information (access control, HR records, historical access trends…)
- Intelligent video surveillance reliably monitors personnel flow, processes and procedures
- Integrated RFID functionality with Smart Access Cards for multiple tasks (e.g. encrypting emails, paying lunch in the cafeteria…)
- All can be integrated into a Danger Management System
- Performance testing and record keeping to document compliance of your laboratories, fume hoods and bio safety
- The solution supports requirements of the European standard EN 14175
- Compliant with global GxP standards as well as local regulations
- Application of regulatory compliance such as Good Laboratory Practices (GLP) and Electronic Records and Electronic Signatures (ER/ES)
- Guarantee of efficient and effective validation including user training and services during operation as well as maintaining the validated status.

## Possible Questions

### Part B (Two marks)

1. Define infection.
2. Define disease.
3. Define stmptoms.
4. Define pathogen.
5. What is a parasite?
6. Define saprophytes.
7. Explain the commensals.
8. Define epidemic.
9. Define endemic
10. Define pandemic.

### Part C (Eight marks)

1. Explain the role of investigation of epidemic.
2. Explain in details about the types of carriers.
3. Comment on primary, secondary and re infection.
4. Write about the importance of quality control?
5. Explain role of QA.
6. Give the role of QC.
7. Explain in detail about the QC and microbiology.
8. Comment on infectious disease cycle.
9. Explain the types of infections.
10. What are the methods of infection?
11. Give the various sources of infection.
12. What are the steps in infectious disease cycle?

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS: I M.Sc MB** **COURSE NAME: MEDICAL BACTERIOLOGY**

**COURSE CODE: 19MBP202** **UNIT: II** **BATCH-2019-2021**

| S.No | Unit II | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | In Greek 'pathos means _____. | Suffering | violence | disease | infection | Suffering |
| 2 | The lodgement and multiplication of a parasite in or on the loscies of a host constitute _____ | disease | infection | immunity | parasitism | infection |
| 3 | Initial infection with a parasite in a host is termed _____ | primary | secondary | re-infection | nosocomial | primary |
| 4 | Subsequent infection by the same parasite in the host is termed _____ infection. | Primary | Secondary | Re-infection | Iatrogenic | Re-infection |
| 5 | When a new parasite sets up an infection in a host whose resistance is lowered by a preexisting infectious disease, this is termed _____ infection. | Primary | Secondary | Re-infection | Iatrogenic | Secondary |
| 6 | Infection or sepsis at localized sites is called _____ infection | Primary | Secondary | Focal | Iatrogenic | Focal |
| 7 | In a patient already suffering from a disease, a new infection is set up from another host or external source it is termed _____ infection. | Cross | Focal | Re-infection | Nosocomial | Cross |
| 8 | Cross infection occurring in hospitals are called ___ infection | Cross | Focal | . Re-infection | | Nosocomial |
| 9 | Physicians induced infections are termed _____ infection | Iatrogenic | Focal | Nosocomial | Re-infection | Iatrogenic |
| 10 | When clinical effects are not appaent it is called _____ infection. | Atypical | Inapparent | Subclinical | Clinical | Inapparent |
| 11 | _____ infection is the one in which the typical or characteristic clinical manifestation of the particular infectious disease are not present. | Endogenous | Exogenous | Inapparent | Clinical | Inapparent |
| 12 | Some parasites, following infection may remain in the tissues in a hidden form proliferating and producing clinical disease termed _____ infection. | Endogenous | Exogenous | Latent | Atypical | Latent |

| | | | | | | |
|---|---|---|---|---|---|---|
| 13 | A person who arbours the pathogenic microorganism without suffering from any ill effects because of it is called _____. | Patient | Carrier | Healthy person | Immunodeficient person | Carrier |
| 14 | A person one who harbours the pathogen but has never suffered from the disease caused by the pathogen is called _____. | Healthy carrier | Convalescent carrier | Contact carrier | Paradoxical carrier | Healthy carrier |
| 15 | A person who have recovered from the disease and continous to harbour the pathogen inside the body is called _____ carrier. | Healthy | Convalescent | Contact | Paradoxical | Convalescent |
| 16 | The _____ carrier state lasts than six months | Acute | Temporary | Chronic | Healthy | Temporary |
| 17 | The term _____ carrier is applied to a person who acquires the pathogen from a patient. | Acute | Temporary | Contact | Paradoxical | Contact |
| 18 | The _____ carrier state lasts for several years. | Acute or chronic | Temporary | Contact | Paradoxical | Acute or chronic |
| 19 | The term _____ carrier refers to a person who acquires the pathogen from another carrier. | Healthy | Convalescent | Contact | Paradoxical | Paradoxical |
| 20 | Infectious diseases transmitted from animals to human being are called _____. | Zoonosis | Anoosis | Xanthosis | Phytosis | Zoonosis |
| 21 | When the pathogen multiplies in the body of the vector and then transmits the infection to humans are called _____ vector. | Mechanical | Biological vector | Healthy | Contact | Biological vector |
| 22 | Some pathogens are able to cross the placental carrier and infect the fetus in uterus. This is known as _____ transmission. | Congenital | Intracelaine | Vertical | Horizontal | Vertical |
| 23 | _____ is generally employed to refer to the ability of a microbial species to produce diseases. | Infection | Immunity | Pathogenicity | Virulence | Pathogenicity |
| 24 | _____ is applied to the same property in a strain of microorganism to produce disease. | Infection | Immunity | Pathogenicity | Virulence | Virulence |
| 25 | _____ is the science that evaluates the occurrence, determinants, distribution and | Epidemiology | Oncology | Infection | Physiology | Epidemiology |

| | | | | | | |
|---|---|---|---|---|---|---|
| | control of health and disease in a defined human population. | | | | | |
| 26 | An individual who practices epidemiology is an _____. | Epidemiologist | Scientist | Investigator | Environmentalist | Epidemiologist |
| 27 | A _____ is an impairement of the normal state of an organism or any of its components that hinders the performance of vital components. | Health | Disease | Infection | Immunity | Disease |
| 28 | _____ is the condition in which the organism performs its vital functions normally. | Health | Disease | Infection | Immunity | Infection |
| 29 | When a disease occurs occasionally and at irregular intervals in a human population it is a _____ disease. | Epidemic | Endemic | Sporadic | Pandemic | Endemic |
| 30 | When the disease occurs at a steady low-level frequency at a moderately regular interval it is an ____ disease. | Epidemic | Endemic | Sporadic | Pandemic | Endemic |
| 31 | _____ disease gradually increase in the occurrence frequency beyond the endemic level but do not to the epidemic level. | hyperendemic | Epidemic | Endemic | Pandemic | hyperendemic |
| 32 | An_____is a sudden increase in the occurrence of a disease above the expected level. | Epidemic | Endemic | sporadic | Pandemic | Pandemic |
| 33 | The first case in an epidemic is called_____ | index case | infection | disease | outbreak | index case |
| 34 | A sudden unexpected occurrence of a disease in a limited segment of population is called_____ | index case | Infection | disease | outbreak | outbreak |
| 35 | A_____is an increase in disease occurrence within a large population over a very wide region. | epidemic | Endemic | Pandemic | Sporadic | Pandemic |
| 36 | The factors that influence the frequency of a disease in an animal population is known as | epizootiology | Zootiology | Epidemiology | Entamology | epizootiology |
| 37 | Moderate prevalance of a disease in animals is termed _____ | epizootic | enzootic | panzootic | zoonoses | enzootic |

| | | | | | | |
|---|---|---|---|---|---|---|
| 38 | A sudden outbreak of disease in animals is called _____ | epizootic | enzootic | panzotic | zoonoses | panzotic |
| 39 | A wide dissemination of aniomals is callled _____ | epizoolic | enzoolic | panzootic | zoonoses | zoonoses |
| 40 | Animal disease that can be transmitted to humans are termed _____ | epizootic | enzootic | panzootic | zoonoses | zoonoses |
| 41 | A _____ period is the period between pathogen entry and the expression of signs and symptoms. | incubation | survival | lag | log | incubation |
| 42 | The _____ is the period in which ther is an onset of sign and symptoms. | incubatiob period | prodromal stage | survival period | death | prodromal stage |
| 43 | The _____ represents the events in the form of an intriguing epidemiological mystery story | infectious disease cycle | disease | infection | none | infectious disease cycle |
| 44 | _____ is an illness caused by a pathogen or its products that has been transmitted from an infected person or a reservoir. | communicable disease | epidemic | epidemic | none | communicable disease |
| 45 | The _____ is the time during which the source is infectious or is disseminating the pathogen. | incubation period | the period of infectivities | predormal stage | none | predormal stage |
| 46 | The water used in the lab is tested by _____ department. | Quarentum | GTL | Materials section | Dispatch | GTL |
| 47 | The Goods purchased is stored at_____ | Dispatch section | Materials section | Quarentum section | Stock section | Quarentum section |
| 48 | The water used in laboratory is purified by _____ filter. | Catridge | Pad | Sinted glass | Earthern ware | Catridge |
| 49 | The monitoring of the lab environment is done by_____. | QA | QC | GTL | QB | QC |
| 50 | The microbial load of lab environment is tested by_____method. | Settle plate | Pour plate | Spread plate | Streak plate. | Settle plate |
| 51 | The AHU stands for_____ | Aerosol handling unit | Air handling unit | Atmosphere handling unit | Auto handling unit | Air handling unit |
| 52 | _____ controls the microbes in air. | Air pressure | a. Aerosol | Atmosphere | oxygen | Air pressure |
| 53 | The circulating air is filtered in lab by | Pad | Candle | HEPA | Catridge | HEPA |

| | | | | | | |
|---|---|---|---|---|---|---|
| | _____ filter. | | | | | |
| 54 | The discard materials to be_____ before disposal. | Dissuaded | Dispenced | Decontaminated | sterilized | Decontaminated |
| 55 | Quality control checks the quality of the product based on the norms of_____ | QA | QC | GTL | QB | QA |
| 56 | The proper disposal of biologicals helps to prevent _____ | Epidemic | Endemic | Pandemic | Randamic | Epidemic |
| 57 | There is no single reliable test available to determine efficiency of a_____. | Antibiotics | Antiseptics | Disinfectants | Diluent. | Disinfectants |
| 58 | In the Rideal walker test typhoid bacilli is tested with _____ | Phenol | Boron | Acid | Alcohol | Phenol |
| 59 | _____ is a product ketane and formaldehyde. | . HCL | BPL | TNT | SDS | BPL |
| 60 | QA stands for _____ | Quality assurance | Quality association | Quality acceptance | Quality abolision. | Quality assurance |

**UNIT-III**
**SYLLABUS**

Morphology, cultural characteristics, antigenic property, pathogenecity, laboratory diagnosis and treatment.*Staphylococcus* sp.,*Streptococcus*sp., *Bacillus* sp., *Corynebacterium*sp., *Clostridium sp. Mycobacterium* sp.

**Introduction to Gram positive organisms**

Gram-positive bacteria are those that are stained dark blue or violet by Gram staining. This is in contrast to Gram-negative bacteria, which cannot retain the crystal violet stain, instead taking up the counterstain (safranin or fuchsine) and appearing red or pink. Gram-positive organisms are able to retain the crystal violet stain because of their thick peptidoglycan layer, which is superficial to the cell membrane. This is in contrast to Gram-negative bacteria, which may have a thick or thin peptidoglycan layer that is located between two cell membranes.

## *Staphylococcus Sp.,*

**Morphology, cultural characteristics of *Staphylococcus***

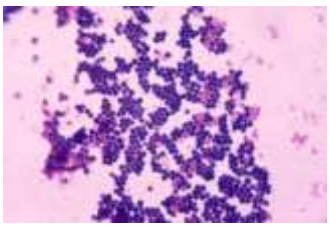
*Staphylococcus aureus* on Columbia agar with 5% defibrinated sheep blood (Bio-Rad™). Individual colonies on agar are round, convex, and 1-4 mm in diameter with a sharp border. On blood agar plates, colonies of *Staphylococcus aureus* are frequently surrounded by zones of clear beta-hemolysis. The golden appearance of colonies of some strains is the etymological root of the bacteria's name; aureus meaning "golden" in Latin.

Methicillin-resistant strains of *Staphylococcus aureus* (i.e. MRSA) often have only weak or no beta-hemolysis and special cultivation media with oxacillin, mannitol and NaCl for their isolation are used. MRSA is able to grow on this media and produce colonies of certain color, depending on used pH indicator.

**Antigenic property of *Staphylococcus***

**Toxins (hemolysins)** – cytolytic, membrane-damaging toxins

- Alpha toxin (hemolysin) – 34 kD protein; principal hemolysin expressed by human strains of *S. aureus*. Toxin monomer binds to cell surface heptamer formation transmembrane channels. Hydrophobic molecule that inserts within the cell membrane of susceptible cells, forming pores. Most active against rabbit erythrocytes, but also active against human platelets. Inject alpha toxin IV -> hemolysis, dermonecrosis, lethality
- Beta toxin (hemolysin) produced commonly by animal strains of *S. aureus* and only by 10 – 20% of human isolates. Beta toxin is a sphingomyelinase C enzyme – catalyzes hydrolysis of membrane phospholipids.
- Gamma toxin (hemolysin). Mode of action – unknown.
- Delta toxin (hemolysin) is produced by most human strains of *S. aureus*. 10,000 to 100,000X less toxic than alpha toxin. Has detergent-like properties.



**Microscopic view of *Staphylococcus***

**Pathogenecity of *Staphylococcus***

Extracellular virulence factors:

**Enzymes:**

- Coagulases:
  - A. bound (**clumping factor**)
  - B. free (**coagulase-reacting factor**)
- Hyaluronidase: "spreading factor" of *S. aureus*
- Nucleases: *S. aureus*; Cleaves DNA (Dnase) and RNA (Rnase)
- Fibrinolysin: staphylokinase
- Lipases: esterases
- Penicillinase

**Exotoxins:**

**Cytotoxins** (hemolysins): cytopathic and cytolytic for a broad range of affected cells, including erythrocytes,leukocytes, macrophages, hepatocytes, lymphocytes, lymphoblasts, fibroblasts, ne utrophils and platelets

- Alpha toxin
- Beta toxin: sphingomyelinase C (phospholipase)
- Delta toxin: detergent-like activity
- Gamma toxin: hemolytic activity
- Leukocidin
- Enterotoxins (A to E)
- Exfoliative toxin (epidermolytic toxin or exfoliatin) (A and B)
- Toxic shock syndrome toxin-1 (TSST-1) (formerly pyrogenic exotoxin C)
- Somatic virulence factors:
- Slime layer (capsule)

**Protein A**: binds Fc receptors of IgG , Teichoic acid: binds fibronectin

**Laboratory diagnosis**

Depending upon the type of infection present, an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification by using biochemical or enzyme-based tests. A Gram stain is first performed to guide the way, which should show typical Gram-positive bacteria, cocci, in clusters. Second, the isolate is cultured on mannitol salt agar, which is a selective medium with 7–9% NaCl that allows *S. aureus* to grow, producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH.

Furthermore, for differentiation on the species level,catalase (positive for all *Staphylococcus* species),coagulase (fibrin clot formation, positive for *S. aureus*),DNAse (zone of clearance on Dnase agar), lipase (a yellow color and rancid odor smell), and phosphatase (a pink color) tests are all done. For staphylococcal food poisoning, phage typing can be performed to determine whether the staphylococci recovered from the food were the source of infection.

**Treatment and Prevention:**

- Drain infected area
- Deep/metastatic infections: semi-synthetic penicillins; cephalosporins, erythromycin or clindamycin
- Endocarditis: semi-synthetic penicillin plus an aminoglycoside
- Carrier status prevents complete control
- Proper hygiene, segregation of carriers from highly susceptible individuals
- Good aseptic techniques when handling surgical instruments

- Control of nosocomial infections

## *Streptococcus Sp.,*

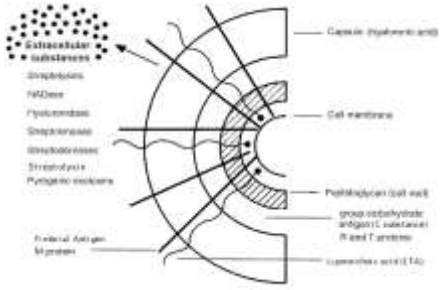
### Morphology of *Streptococcus*

*Streptococci* are Gram-positive, nonmotile, nonsporeforming, catalase-negative cocci that occur in pairs or chains. Older cultures may lose their Gram-positive character. Most streptococci are facultative anaerobes, and some are obligate (strict) anaerobes. Most require enriched media (blood agar). Group A streptococci have a hyaluronic acid capsule.

**Microscopic view of *Streptococcus***

### *Classification and Antigenic Types*

Streptococci are classified on the basis of colony morphology, hemolysis, biochemical reactions, and (most definitively) serologic specificity. They are divided into three groups by the type of hemolysis on blood agar: β-hemolytic (clear, complete lysis of red cells), α hemolytic (incomplete, green hemolysis), and γ hemolytic (no hemolysis). Serologic grouping is based on antigenic differences in cell wall carbohydrates (groups A to V), in cell wall pili-associated protein, and in the polysaccharide capsule in group B streptococci.



### Pathogenecity of *Streptococcus*

Group A streptococci are model extracellular gram-positive pathogens responsible for pharyngitis, impetigo, rheumatic fever, and acute glomerulonephritis. A resurgence of invasive streptococcal diseases and rheumatic fever has appeared in outbreaks over the past 10 years, with a predominant M1 serotype as well as others identified with the outbreaks. emm (M protein) gene sequencing has changed serotyping, and new virulence genes and new virulence regulatory networks have been defined. The emm gene superfamily has expanded to include antiphagocytic molecules and immunoglobulin-binding proteins with common structural features. At least nine superantigens have been characterized, all of which may contribute to toxic streptococcal syndrome. An emerging theme is the dichotomy between skin and throat strains in their epidemiology and genetic makeup. Eleven 3dhesions have been reported, and surface plasmin-binding proteins have been defined. The strong resistance of the group A *Streptococcus* to phagocytosis is related to factor H and fibrinogen binding by M protein and to disarming complement component C5a by the C5a peptidase. Molecular mimicry appears to play a role in autoimmune mechanisms involved in rheumatic fever, while nephritis strain-associated proteins may lead to immune-mediated acute glomerulonephritis. Vaccine strategies have focused on recombinant M protein and C5a peptidase vaccines, and mucosal vaccine delivery systems are under investigation.

**Laboratory diagnosis of *Streptococcus***

Infective stages may be present in CSF, joint aspirates, blood, abscesses, aerosols, faeces, and urine.

**Treatment**

Treatment of abscesses usually does not need antibiotic therapy; appropriate drainage is usually sufficient. Proper antibiotic therapy is required for more serious infections.

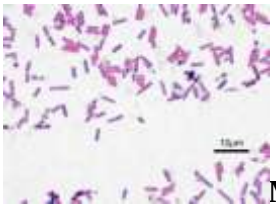
## *Bacillus Sp.,*

**Morphology of Bacillus**

The word ***bacillus*** (plural ***bacilli***) may be used to describe any rod-shaped bacterium, and such bacilliare found in many different taxonomic groups of bacteria. However, the name Bacillus, capitalized and italicized, refers to a specific genus of bacteria. The name Bacilli, capitalized but not italicized, can also refer to a more specific taxonomic class of bacteria that includes two orders, one of which contains the genus *Bacillus*. Bacilli are usually solitary, but can combine to form diplobacilli, streptobacilli, and palisades.

**Pathogenecity of *Bacillus***

*B. anthracis* possesses a capsule that is antiphagocytic and is essential for full virulence. The organism also produces three plasmid-coded exotoxins: edema factor, a calmodulin- dependent adenylate cyclase, causes elevation of intracellular cAMP, and is responsible for the severe edema usually seen in *B. anthracis* infections; lethal toxin is responsible for tissue necrosis; protective antigen (so named because of its use in producing protective anthrax vaccines) mediates cell entry of edema factor and lethal toxin.



**Microscopic view of *Bacillus***

**Laboratory diagnosis**

The diagnosis of type based on morphology, affinity dye (sometimes very difficult to determine because some Gram-positive species appear only in old cultures a few hours), the study of respiratory type and the identification of a spore. As pointed out Logan and Turnbull, it is essential to establish that the suspect colonies are made up of gram-positive bacteria, sporulated and capable of growing in aerobically.

**Prevention and treatment**

A number of anthrax vaccines have been developed for preventive use in livestock and humans. Infections with *B. anthracis* can be treated with β-lactam antibiotics such as penicillin, and others which are active against Gram-positive bacteria.[19] Penicillin-resistant *B. anthracis* can be treated with fluoroquinolones such as ciprofloxacin or tetracycline antibiotics such as oxycycline.

## *Corynebacterium Sp.,*

**Morphology of Corneybacterim**

***Corynebacterium diphtheriae*** is a pathogenic bacterium that causes diphtheria. It is also known as the **Klebs-Löffler bacillus**, because it was discovered in 1884 by German bacteriologists Edwin Klebs (1834 – 1912) and Friedrich Löffler (1852 – 1915). Four subspecies are recognized: *C. diphtheriae mitis*, *C. diphtheriae intermedius*, *C. diphtheriae gravis*, and *C. diphtheriae belfanti*. The four subspecies differ slightly in their colonial morphology and biochemical properties, such as the ability to metabolize certain nutrients, but all may be toxigenic (and therefore cause diphtheria) or non-toxigenic.

*Corynebacterium diphtheriae* produces Diphtheria toxin which alters protein function in the host by inactivating elongation factor (EF-2). This causes pharyngitis and 'pseudomembrane' in the throat.

The diphtheria toxin gene is encoded by a bacteriophage found in toxigenic strains, integrated into the bacterial chromosome.

**Culture characteristic**

In order to accurately identify *C. diphtheriae*, a Gram stain is performed to show gram-positive, highly pleomorphic organisms with no particular arrangement. Special stains like Alberts's stainand Ponder's stain are used to demonstrate the metachromatic granules formed in the polar regions. The granules are called as polar granules, Babes Ernst Granules, Volutin, etc. An enrichment medium, such as Löffler's medium, is used to preferentially grow *C. diptheriae*. After that, use a differential plate known as tellurite agar, which allows all *Corynebacteria*(including *C. diphtheriae*) to reduce tellurite to metallic tellurium. The tellurite reduction iscolormetrically indicated by brown colonies for most *Cornyebacteria* species or by a black halo around the *C. diphtheriae* colonies.

**Microscopic view of *Corneybacterium***

**Antigenic Property**

*Corynebacterium diphtheriae* is a nonmotile, noncapsulated, club-shaped, Gram-positive bacillus. Toxigenic strains are lysogenic for one of a family of corynebacteriophages that carry the structural gene for diphtheria toxin, *tox. Corynebacterium diphtheriae* is classified into biotypes (mitis, intermedius, and gravis) according to colony morphology, as well as into lysotypes based upon corynebacteriophage sensitivity. Most strains require nicotinic and pantothenic acids for growth; some also require thiamine, biotin, or pimelic acid. For optimal production of diphtheria toxin, the medium should be supplemented with amino acids and must be deferrated.

**Pathogenesis *Corynebacterium***

Asymptomatic nasopharyngeal carriage is common in regions where diphtheria is endemic. In susceptible individuals, toxigenic strains cause disease by multiplying and secreting diphtheria toxin in either nasopharyngeal or skin lesions. The diphtheritic lesion is often covered by a pseudomembrane composed of fibrin, bacteria, and inflammatory cells. Diphtheria toxin can be proteolytically cleaved into two fragments: an N-terminal fragment A (catalytic domain), and fragment B (transmembrane and receptor binding domains). Fragment A catalyzes the $NAD^+$-dependent ADP-ribosylation of elongation factor 2, thereby inhibiting protein synthesis in eukaryotic cells. Fragment B binds to the cell surface receptor and facilitates the delivery of fragment A to the cytosol.

**Diagnosis**

Clinical diagnosis depends upon culture-proven toxigenic *C. diphtheriae* infection of the skin, nose, or throat combined with clinical signs of nasopharyngeal diphtheria (e.g., sore throat, dysphagia, bloody nasal discharge, pseudomembrane).

**Control**

Immunization with diphtheria toxoid is extraordinarily effective. Diphtheria patients must be promptly treated with antitoxin to neutralize circulating diphtheria toxin.

## *Clostridium Sp.,*

**Morphology of Clostridium**

Most Clostridium species decompose proteins or form toxins and some do both. Their natural habitat is the soil or intestinal tract as saprophytes. The important pathogenic species are:

- *Clostridium botulinum*: Causes botulism

- *Clostridium tetani*: Causes tetanus
- *Clostridium perfringens*: Causes gas gangrene
- Large anaerobic gram positive motile rods.
- The spore is usually wider than the rods.
- Spores are placed centrally, terminally, or subterminally according to the genus.

**Cultural characteristics**

Anaerobic culture conditions are established by one of the following:

Agar plates or culture tubes are placed in air tight jar from which air is removed and replaced by N and CO2.

- Fluid media contain either:
- Fresh animal tissue (chopped meat)
- Reducing agent (Thioglycolate)

**Gram's staining view of *Clostridium tetani***

**Colony forms**

*Clostridium perfringens*: Large raised colonies with entire margins.

*Clostridium tetani* : Smaller colonies with fine filaments.

Most Clostridia produce a zone of hemolysis on blood agar.

**Growth characteristics of anaerobic microorganisms are:**

- Unable to utilize O2 as the final oxygen acceptor.
- Lack of cytochrome and cytochrome oxidase.
- Unable to break down hydrogen peroxide H2O2 because they lack catalase of peroxidase so $H_2O_2$ will accumulate to toxic conc. in the presence of $O_2$.

***Clostridium botulinum***

It causes botulism, infant's botulism, and rarely wound infection. It is found in soil and animal feces. The spores are subterminal highly resistant to heat. They resist boiling 3-5 hours. This resistance is diminished at acidic pH and salt. It produces toxin during life and autolysis of bacteria.

**Pathogenesis of *Clostridium***

Botulism is intoxication. It results from ingestion of food in which *Clostridium botulinum* spores germinate and produce toxins under anaerobic conditions. These foods are spiced, smoked vacuum-packed, or canned alkaline foods (if eaten without smoking). The toxin acts by blocking the release of acetyl choline at synapses and neuromuscular junctions causing flaccid paralysis. Patients who recover don't develop an antitoxin in the blood.

**Symptoms[ within 18 – 24 hours ]**

- Visual disturbances
- Inability to swallow
- Speech difficulty
- Respiratory paralysis or cardiac arrest

**Lab diagnosis**

- Toxin can be detected in the patient serum and left over food.
- Mice are injected with the specimen and then neutralized by injections of antitoxin.
- Culture of food remains of its growth test for toxin production.

- Toxin is tested by hemoagglutination or radioimmunoassay (RIS).

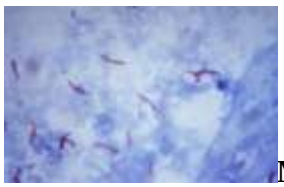
**Treatment**

- IV administration of antitoxin (trivalent antitoxin of types A, B, and E).
- Adequate ventilation by mechanical respirator. This will reduce mortality form 65% to 25%
- Infant botulism is recovered with supportive therapy alone.

## *Mycobacterium Sp.,*

**Morphology of Mycobacterium**

Typical organisms: In tissue, tubercle bacilli are thin straight rods with variable morphology from one species to another. Mycobacteria cannot be classified as gram +ve or -ve. They are characterized by 'acid-fastness i.e. 'acid-alcohol' quickly decolorizes all bacteria except the mycobacteria, acid- fastness depends on the integrity of the waxy envelope .The Ziehl-Neelsen technique of staining is employed for identification of acid-fast bacteria.



**Microscopic view of *Mycobacterium***

**Cultural characteristic of *Mycobacterium***

There are three general formulations that can be used for both the nonselective and the selective media:-

1. Semi-synthetic agar media (eg, Middlebrook 7H10 and 7H11) contain salts, vitamins cofactors, oleic acid, albumin, catalase, glycerol, glucose, and malachite green. Large inocula yield growth on these media in several weeks. These media may be less sensitive than other media for primary isolation of mycobacteria.
2. Inspissated egg media (eg, Lowenstein-Jensen) contain salts, glycerol, and complex organic substances (eg, fresh eggs, egg yolks, potato flour, and other ingredients).Malachite green is included to inhibit other bacteria. Small inocula in specimens from patients will grow on these media in 3-6 weeks. These media with added antibiotics are used as selective media.
3. Broth media: broth media (eg, Middlebrook 7H9 and 7H12) support the proliferation of small inocula. Mycobacteria grow in clumps or masses because of the hydrophobic character of the cell surface, and added antibiotics.

**Growth characteristics:**

- Mycobacteria are obligate aerobes.
- Increased Co2 tension enhances growth.
- Biochemical activities are not characteristics, and the growth rate is much slower than that of most bacteria.
- Saprophytic forms tend to grow more rapidly, to proliferate well at 22-33 c To produce more pigment, and to be less acid-fast than pathogenic forms.

**Pathogenesis**

Mycobacteria in droplets are inhaled and reach the alveoli. The disease results from establishment and proliferation of virulent organisms and interactions with the host. Resistance and hypersensitivity of the host greatly influence the development of the disease.

**Diagnostic laboratory tests**

A positive tuberculin test does not prove the presence of active disease due to tubercle bacilli.

A. Specimens: consist of fresh sputum, gastric washings, urine, pleural fluid, C5F, joint fluid, biopsy material, blood, or other suspected material.
B. Decontamination and Concentration of Specimens: Specimens from sputum with NaOH, neutralized with buffer, and concentrated by centrifugation. Used for acid-fast stains and for culture.
C. Smears:

- Examined for acid-fast bacilli by Ziehl-Neelsen staining.
- Antigen Detection, serology and anti-gene detection (PCR)
- The polymerase chain reaction holds great promise for the rapid and direct detection of M. tuberculosis in clinical specimens- the PCR test is approved for this use.

**Antigen Detection, serology and anti-gene detection (PCR)**

The polymerase chain reaction holds great promise for the rapid and direct detection of M.tuberculosis in clinical specimens- the PCR test is approved for this use.

**Treatment**

- Prompt and effective treatment of patients with active tuberculosis
- Drug treatment of asymptomatic tuberculin-positive persons (eg, children)-receive immunosuppressive drugs.
- Nonspecific factors may reduce host resistance include starvation, gastrectomy, and suppression of cellular immunity by drugs.
- Immunization: Various living avirulent tubercle bacilli, particularly BCG (Bacillus Calmette-Guerin, an attenuated bovine organism). Vaccination is a substitute forprimary infection with virulent tubercle bacilli without the danger inherent in the latter given to children.
- The eradication of tuberculosis in cattle and the pasteurization of milk have greatly reduced M.bovis infections.

**Possible Questions**

**Part B (Two marks)**

1. Define pyogen.
2. Write about the morphology of S.aureus?
3. What are the cultural characteristics of Staphylococcus?
4. Write about the morphology of *Corynebacterium*?
5. Write about the morphology of *Streptococcus*?
6. Give the morphology of *Bacillus*.
7. Give the morphology of *Clostridium*.
8. Write about the morphology of *Mycobacterium*?
9. Comment on the biochemical property of *Streptococcus*.
10. Comment on the biochemical property of *Bacillus*.
11. Explain the antigenic property of *Bacillus.*

**Part C (Eight marks)**

1. Comment on the biochemical property of Staphylococcus.
2. Discuss the antigenic property of Staphylococcus.
3. Laboratory diagnosis of *S.aureus*.
4. Comment on the biochemical property of *Corynebacterium*.
5. Discuss the antigenic property of *Corynebacterium.*
6. Laboratory diagnosis of *Corynebacterium*.
7. Discuss the antigenic property of *Streptococcus*.
8. Laboratory diagnosis of *Streptococcus*.
9. Discuss the cultural characteristics of *Bacillus*.
10. Outline the Laboratory diagnosis of *Bacillus*.
11. Discuss the cultural characteristics of *Clostridium.*
12. Comment on the biochemical property of *Clostridium*.
13. Explain the antigenic property of *Clostridium.*
14. Outline the Laboratory diagnosis of *Clostridium*.
15. Comment on the biochemical property of *Mycobacterium.*
16. Discuss the antigenic property of *Mycobacterium.*
17. Write about the Laboratory diagnosis of *Mycobacterium*?

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS: I M.Sc MB** **COURSE NAME: MEDICAL BACTERIOLOGY**

**COURSE CODE: 19MBP202** **UNIT: III** **BATCH-2019-2021**

| S.No | Unit III | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | species often resemble Staphyloccus | micrococcus | streptococcus | lactococcus | staphylococcus | streptococcus |
| 2 | established the pathogenic role of staphylocci in | pasteur | emil von behring | jenner | alexander ogston | alexander ogston |
| 3 | staphylococci can grow in the presence of 10% or more of . | peptone | beef extract | NaCl | lactose | NaCl |
| 4 | is the selective medium for isolation of Streptococcus pyogenes | blood agar | PLET | nutrient agar | crystal violet blood agar | crystal violet blood agar |
| 5 | local infection of superficial layers of skin, especially in children is called | impetigo | pustule | hemorrhage | meningitis | impetigo |
| 6 | The test is used for the identification of Staphyloccus aureus isolates | slide coagulase test | tube coagulase test | Grams test | serum test | slide coagulase test |
| 7 | Streptococcus pyogenes are more sensitive to than other species. | amoxycillin | penicillin | erythromycin | bacitracin | bacitracin |
| 8 | the disease common in dockworkers carrying loads of skin and animals was known as | hide porter's disease | gas gangrene | edeme | toxemia | hide porter's disease |
| 9 | _____ strains usually secrete both coagulase and clumping factor. | micrococcus | streptococcus | staphylococcus aureus | E.coli | staphylococcus aureus |
| 10 | Vegetative cells of Bacilli are destroyed at C in 30 min | 60C | 40C | 20C | 10C | 60C |
| 11 | The spores of Bacillus anthracis were isolated from the infected soil after _____ years. | 20 | 30 | 40 | 60 | 60 |
| 12 | Lepra bacilli have been found to remain viable in moist soil for days | 100 | 80 | 46 | 20 | 46 |
| 13 | The Lepra bacilli seen in large numbers as golbi inside Lepra cells is known as _____ | Common disease | Multibaciliry disease | Gas gangrene | Symptomatic disease | Multibaciliry disease |

| | | | | | | |
|---|---|---|---|---|---|---|
| 14 | was the first effective chemotherapeutic agent against leprosy | rifampicin | clofazimine | ethionamide | dapsone | dapsone |
| 15 | _____ vaccine was used to prevent leprosy. | BCG | Polio vaccine | Leprosy vaccine | Rabies vaccine | BCG |
| 16 | The Lepra bacillus was first observed by _____ | Jenner | Behring | Pasteur | Hansen | Hansen |
| 17 | The diphtheria bacillus was first observed by _____. | Pasteur | Hansen | Klebs | Loeffler | Klebs |
| 18 | Typing in Pneumococcus may be carried out by reaction. | serum typing | Quellung | Bamboo-stick | stickland | Quellung |
| 19 | Corynebacterium exists in a _____ morphology | Rod | Cocci | Varied | Spindle shaped | Varied |
| 20 | On repeated subculture Pneumococci undergo a variation. | smooth-rough | rough-smooth | smooth | rough | smooth-rough |
| 21 | is always a secondary infection caused by any serotype of Pneumococcus. | meningitis | hemorrhage | paralysis | Broncho-pneumonia | Broncho-pneumonia |
| 22 | The strain used to produce the diphtheria toxin is _____ | Bacillus Calmette | Park Williams 8 Strain | Park Williams 5 Strain | Bacillus Calmette 8 strain | Park Williams 8 Strain |
| 23 | The diagnostically important constant property of Pneumococcus is | Bile solubility | serum solubility | symptoms | pathogeicity | Bile solubility |
| 24 | The incubation period of Lepra bacilli ranges from years | 5 to 6 | 2 to 5 | 4 to 9 | 1 to 3 | 2 to 5 |
| 25 | The BCG vaccine used to prevent leprosy was suggested by | Fernandez | Pasteur | Jenner | Hansen | Fernandez |
| 26 | Preventation of anthrax in animals is aided by | prophlaxix | treatment | active immunization | antibiotics | active immunization |
| 27 | reaction is useful for the primary diagnosis of anthrax in animals. | M'Fadyean's | Quellung | Nagler | immunization | M'Fadyean's |
| 28 | Staphylococci are lysed under the influence of the drug | Penicillin | streptomycin | amoxycillin | dapsone | Penicillin |
| 29 | is a typical of Staphycoccal infection | pus | edema | focal suppuration | impetigo | focal suppuration |

| | | | | | | |
|---|---|---|---|---|---|---|
| 30 | Streptococcal sore throat is commonly called | sore throat | Strep.throat | infection | disease | sore throat |
| 31 | named the strains Staphylococcus aureus and S.albus. | Jenner | Behring | Rosenbach | Klebs | Rosenbach |
| 32 | Typical Staphylococci are seen in the stained smears of | pus | blood | stool | urine | pus |
| 33 | is the selective medium used for the isolation of Bacillus anthracis | PLET | blood agar | Mac-conkey | PDA | PLET |
| 34 | In cultures Bacillus were arranged ene to end in a long chain gives a appearance | chain | Bamboo-stick | cluster | thread-like | Bamboo-stick |
| 35 | The of Corynebacterium was described by Von Behring. | exotoxin | antitoxin | size | shape | antitoxin |
| 36 | type of complications are most common in gravis type of Corynebacterium. | fever | headache | paralytic | nausea | paralytic |
| 37 | Food poisoningby Clostridium perfringens is usually caused by cold or warmed up dish. | fish | egg | soup | meat | meat |
| 38 | Pneumococci were first noticed by . | Nagler | Robert Hooke | Pasteur and Sternberg | Kleb and Loeffler | Pasteur and Sternberg |
| 39 | The toxin produced by virulent strain of diphtheria is known as . | exotoxin | endotoxin | antitoxin | toxin | exotoxin |
| 40 | The drug of choice for treating gas gangrene is . | Penicillin | streptomycin | metranidazole | Amoxycillin | metranidazole |
| 41 | The irregularly stained boat or leaf shaped Clostridium septicum is known as . | citron bodies | pleomorphs | irregular bodies | boat bodies | pleomorphs |
| 42 | The arrangement of diphtheria bacilli at various angles to each other resembling the letters V or L has been called as _____. | V shaped | L shaped | Chinese | Bamboo- stick | Chinese |
| 43 | The causative agent of gas gangrene is ____ | streptococcus | micrococcus | Clostridium perfringens | Corynebacterium | Clostridium perfringens |

| | | | | | | |
|---|---|---|---|---|---|---|
| 44 | Clostridium species can grow well in _____ broth | PLET | Robertson's cooked meat | PDA | Mac-conkey | Robertson's cooked meat |
| 45 | _____ dyes are used to stain Pneumococci. | Acidic dyes | Basic dyes | Neutral dyes | Aniline dyes | Aniline dyes |
| 46 | The most important antigen of Pneumococcus is _____ | Type specific capsular polysaccharide | Type specific Nucleotide | Type specific antigen | Type specific antibody | Type specific capsular polysaccharide |
| 47 | When sputum is not available may be used for culturing Pneumococcus | blood | serum | serum coated laryngeal swabs | egg | serum coated laryngeal swabs |
| 48 | is an antigeniccomponent of the cell wall facilitates adhesion of the cocci to the host cell surface | lipopolysaccharide | teichoic acid | lactate | iron | teichoic acid |
| 49 | Staphylococcus cause_____ | Tumour | Boils | Lesion | Fever | Lesion |
| 50 | Staphylococcus were first observed by_____ | Vonreckling hausen | Ogsten | Passet | Pasteur | Vonreckling hausen |
| 51 | Staphylococcus citreus produce_____ colour | Red | Blue | Brown | Yellow | Yellow |
| 52 | Non virulent Staphylococcus is found in _____ | Water | Hospital | . Skin | Hair | . Skin |
| 53 | Mannitol is fermented by _____ Staphylococcus | Virulant | Avirulant | Commensal | Normal flora | Virulant |
| 54 | Oil paint appearance is seen in_____ medium | Nutrient broth | Nutrient agar | Blood agar | Blood broth | Nutrient agar |
| 55 | In intoxication the disease is caused by _____ | Toxoid | Tetroid | .Enzyme | Toxin | Toxin |
| 56 | Alpha haemolysis means _____ | Complete | Irregular | . Partial | Nolysis | . Partial |
| 57 | Beeta haemolysis means _____ | Complete | Irregular | Partial | Nolysis | Complete |
| 58 | Selective media for Staphylococcus _____ | Manittol salt agar | Mac conkey agar | Blood agar | LJ agar | Manittol salt agar |
| 59 | Streptococcus shows | Alpha | Beta | Gamma | delta | Beta |

| | | | | | | |
|---|---|---|---|---|---|---|
| | _____ type of haemolysis | | | | | |
| 60 | Pneumococcus habitate in the _____ part of human | Liver | Urinary tract | Respiratory tract | Bone marrow | Bone marrow |

**UNIT-IV**
**SYLLABUS**

Morphology, cultural characteristics, antigenic property, pathogenecity, laboratory diagnosis and treatment. *E.coli*, *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Vibrio* sp., *Salmonella* sp., *Shigella*sp.,*Treponema*sp., *Leptospira*sp; *Neisseria* sp. and *Haemophilus*sp.

## *Escherichia coli*

**Morphology**:

- ***Escherichia coli*** commonly abbreviated ***E. coli*** is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms).
- Strains that possess flagella are motile. The flagella have a peritrichous arrangement
- Capsules or microcapsules made of acidic polysaccharides .They may vary in their size. Capsules detected by light microscopy .Microcapsules by serological or chemical techniques

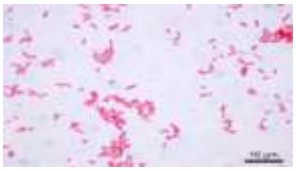


**Fig: Gram negative rods- *E.coli***

**Cultural Characteristics**:

a. *E. coli* is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 microns (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 – 0.7 (μm).
b. Ferments simple sugars such as glucose to form lactic, acetic, and formic acids.
c. The optimum pH for growth is 6.0 to 8.0. However, growth can occur as low as pH 4.3 and as high as 9 to 10 pH
d. Optimal growth temperature is 37°C
e. After 18–24 h of incubation at 37°C, large (2–3 mm), circular, convex, and non pigmented colonies on nutrient and blood agar (Large thick greyish white moist smooth opaque or partially translucent discs )
f. Some strains produce β hemolysis (hemolysin)
g. Grow as large red colonies on MacConkey agar

**Fig: Blood agar (**smooth opaque**)**

**Fig: MacConkey agar (**large red colonies**)**

**Antigenic structure**:

a. Approximately 170 different O antigens have been delineated and some of these are cross-reactive with *Shigella, Salmonella* and *Klebsiella*.
b. Motile strains possess H (flagellar) antigens that can be used for epidemiologic purposes.
c. *Escherichia* also possess K (capsular) antigens similar to the Vi antigen of *Salmonella*.
d. Enterotoxigenic strains may also display colonization factor antigens (CFA/I, CFA/II).

**Pathogenesis**:

The pathogenic *E. coli* within each pathotype may be further classified as virotypes, based on the virulence genes that they possess. A virotype is a particular combination of virulence genes. Important virulence factors encoded by these genes include fimbrial adhesins, enterotoxins, cytotoxins, capsule, and lipopolysaccharide, or LPS. Pathogenic *E. coli* may also be differentiated by serotypinc, based on antigenic differences in the O antigen of the LPS, in the flagellar or H antigens, and the fimbrial or F antigens.

Pathogenic infections include:

a. Enteropathogenic (EPEC)
b. Enteroinvasive (EIEC)
c. Enterotoxigenic (ETEC)
d. Vero cytotoxigenic (VTEC)
e. Diffusely adherent (DAEC)
f. Uropathogenic (UPEC)

**Enteropathogenic *Escherichia coli* (EPEC)**

Pathogenic bacteria contaminating the environment are ingested by susceptible animals and enter the intestinal tract (1). These bacteria possess fimbrial adhesins which mediate adherence to specific receptors on the intestinal epithelial cells (2). Resulting bacterial colonization is found mostly on the jejunal and or ileal mucosa. The adherent bacteria produce enterotoxins which stimulate water and electrolyte loss into the intestinal lumen (3), leading to dehydration and possibly death, and a decreased weight gain in surviving animals (4).

**EIEC (Entero invasive *Escherichia coli)***

EIEC strains are invasive pathogens.The first step in this process is the attachment to the host cell. This induces rearrangements in the cytoskeleton of the cells and eventually leads to phagocytosis of the bacteria. The invasion plasmid antigen (Ipa) D is believed to be involved in adhesion. IpaB and IpaC seem to be essential for phagocytosis and for rupture of the phagocytic vesicle Intercellular spread, which again involves continuous reorganization of the host cell actin filaments, is mediated by two proteins, IcsA (also called VirG) and IcsB, and ultimately leads to cell death.

**VTEC (Verotoxigenic *Escherichia coli*)**

Potentially pathogenic bacteria are ingested by cattle and other ruminants (1) and colonize the intestinal tract, but do not cause any disease in these animals. The bacteria are excreted in the feces and contaminate the environment, including the drinking and swimming water of the human population (2). There may also be contamination of foods such as fruits, vegetables, sprouts, lettuce, and raw milk and juice (3). There may be contamination of the carcass at slaughter, and bacteria will be mixed into ground beef. Persons in direct contact with animals, who are working on farms or in slaughter-houses, may also be contaminated by the bacteria (4). There may also be spread of bacteria from person to person (5). In humans, these bacteria colonize mostly the large intestine and cause similar attaching and effacing lesions to those described for AEEC (6). Bacteria produce their own specific receptor which is injected into the host epithelial cell via a syringe-like bacterial apparatus. A bacterial adhesin then mediates a very intimate

attachment of the bacteria to the cell receptors and bacterial signals stimulate effacement of the microvilli, or brush border, and reorganization of the cell cytoskeleton. The adherent bacteria produce a toxin which is transported across the epithelial cells to the circulation (7). This toxin acts on the endothelial cells of blood vessels, resulting in nonbloody to bloody diarrhea and abdominal cramps (8). There may be a complication of hemolytic uremic syndrome which may lead to acute kidney failure, especially in children.

**Diffusely adherent *Escherichia coli* (DEAC)**

Pathogenic bacteria contaminating the environment are ingested by susceptible animals and enter the intestinal tract (1). Focal to extensive bacterial colonization of small and large intestine is observed (2). Bacteria produce their own specific receptor which is injected into the host epithelial cell via a syringe-like bacterial apparatus. A bacterial adhesin then mediates a very intimate attachment of the bacteria to the cell receptors and bacterial signals stimulate effacement of the microvilli, or brush border, and reorganization of the cell cytoskeleton (3). The adherent bacteria also stimulate epithelial cell degeneration, and infiltration of PMN's in lamina propria. These cell changes may lead to the appearance of diarrhea (4).

**Uropathogenic *Escherichia coli* (UPEC)**

Potentially pathogenic bacteria contaminating the environment are ingested by susceptible animals and enter the intestinal tract or enter via the respiratory tract (chickens) (1). These bacteria are considered to be opportunistic pathogens, as they remain as part of the normal microflora and colonize intestinal, respiratory or other mucosal surfaces, possibly due to fimbrial adhesins (2). When the animal is weakened, such as following a viral infection, due to ingestion of mycotoxins, or when a newborn has not received enough colostrum, bacteria pass more readily through the mucosa to the circulation (3). These internalized bacteria can resist the lethal effects of complement and phagocytes (4) and persist and multiply in the system (5 also in part due to the production of aerobactin. The production of toxins by the bacteria may contribute to tissue damage (6). Release of endotoxins by dead bacteria, may trigger cytokine responses leading to shock and death of the animal (7). In localized infections, there may be bacterial interaction with extracellular matrices, leading to pneumonia, serositis, mastitis, urinary tract infection, etc. (8).

**Clinical Features:**

a. Urinary tract infection- cystitis, pyelonephritis
b. Wound infection- appendicitis, peritonitis
c. Neonatal septicemia and meningitis
d. E.coli-associated diarrheal disease

1. Enteropathogenic E.coli (EPEC)
   - causes outbreaks ofself-limiting infantile diarrhea
   - they also cause severe diarrhea in adults
   - antibiotic tretment shorten the duratin of illness and cure diarrhea
2. Enteroinvasive E.coli (EIEC)
   - Non-motile, non-lactose fermenting E.coli invade the mucosa of the ileum and colon, and causes shigellosis-like dysentery in children in developing
3. Enterotoxigenic E.coli (ETEC)
   - Colonization factor of the organism promote adherence to epithelial cells of small intestine followed by release of enterotoxin which causes toxin-mediated watery diarrhea in infants and young adults.
   - It is an important cause of traveller's diarrhea
   - Antibiotic prophylaxis can be effective but may increase drug resistance

4. Entero haemorrhagic E.coli( EHEC)
   - Cytotoxic verotoxin producing E.coli serotype O157:H7 causes haemorrhagic colitis (severe form of diarrhea), and hemolytic uremic syndrome characterized by acute renal failure, hemolytic anemia and low platelet count

5. Enteroaggressive E.coli ( EAEC)
   - Adhere to human intestinal mucosal cells and produce ST-like toxin and hemolysin, and causes acute and chronic diarrhea in persons in developing countries
   - Produce food-borne illness in developed

**Laboratory diagnosis**:
- **Specimen**: Urine, pus, blood, stool, body fluid
- **Smear:** Gram-negative rods
- **Culture**: Lactose-fermenting mucoid colonies on mac conkey agar and some strains are hemolytic on blood agar.
- **Biochemical reaction**: Produce indole from tryptophancontaining peptone water.Reduce nitrate to nitrite.
- **Serology:** For serotyping (Epidemiologic information)

**Treatment**:
a. Base on antibiotic sensitivity pattern countries and travellers to these countries
b. Antibiotic therapy is not generally recommended unless disease becomes life-threatening. Oral rehydration is the best treatment.
c. New vaccines against fimbrial antigens are possible.

**Prevention and control**:
- **Sanitary:** As with other fecal-oral diseases, proper food handling and personal hygiene are the best means for preventing infection.

**Immunological:** New vaccines against fimbrial antigens are possible.

## *Klebsiella pneumoniae*

**Morphology**:
a. Gram negative rod ,encapsulated, non-motile, non-spore forming, facultative anaerobic
b. opportunistic pathogen belonging to the Enterobacteriaceae
c. Reservoirs -Humans (normal flora of the skin, nasopharynx, oropharynx and GI tract), animals, water,soil
d. Transmission by direct contact,fecal-oral,contaminated fomites

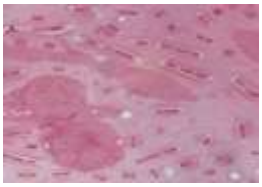


**Fig: *K.pneumoniae* Gram-negative, short rods with parallel sides and rounded ends**.

**Cultural Characteristics**:
a. *K. pneumoniae* consists of straight rods 1 to 2 μm (micrometres) in length with a thick, surrounding capsule. When cultured, this species produces a distinctive yeasty odor and bacterial colonies have a viscous/mucoid appearance.
b. In Nutrient agar– mucoid, circular, convex, small colonies
c. MacConkey agar– mucoid, rose pink
d. Biochemical reactions:

IMViC: - -- + +TSI:Acidbutt/acidslantwithgas production\

e. *K. pneumoniae* is lactose fermenting, H2S- and indole-negative, has a positive Voges-Proskauer (VP) reaction, is capable of growth in KCN and using citrate as a sole carbon source, and is incapable of growth at 10ºC.

**Fig: *K.pneumoniae* on blood agar showed medium sized, grey colonies, which cause no alteration of the blood.**

**Fig: *K.pneumoniae* on Mac Conkey agar plates showing Lactose fermenting colonies.**

**Antigenic structure**:

Members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface. The first, O antigen, is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties.[3] Both contribute to pathogenicity and form the basis for serogrouping

**Pathogenesis**:

This pathogen possesses many virulence factors that allow it to go undetected by the host's immune system and cause infection in a variety of ways. Firstly, this species uses ferric-siderophore receptors of the host to activate their enterobactin-mediated iron-sequestering system, allowing for bacterial growth. Their thick polysaccharide capsule prevents ingestion by phagocytes and their somatic antigens from being detected by the host's antibodies. Also, serum *complement* activation is more difficult with the thick lipopolysaccharide capsule it possesses (Greenwood *et al.*, 2002). In fact, *K. pneumoniae* avoids damage by complement proteins by the extreme length of the molecules comprising the capsule, essentially allowing the lytic C5b-9 (complement) complex to form too far away from the membrane. This prevents opsonization and *membrane attack complex* (MAC) insertion, which leads to lysis of the bacterium .

**Clinical Features:**

Second most common cause of lobar pneumonia and pulmonary abscesses primarily caused by *K.. pneumoniae*

a. wound infections (primarily if burned, caused by all species of *Klebsiella*)
b. cystitis (primarily if urinary catheter, caused by all species of *Klebsiella)*
*c.* second most common cause of septicemia caused by all species of *Klebsiella*

Associated pneumonia

a. Frequently associated with
    i. Necrotic destruction of alveolar spaces
    ii. Production of blood-tinged sputum
b. Can also cause wound, soft tissue, and urinary tract infections

**Laboratory diagnosis**:

The diagnosis of the infection is done in two ways

- Gram stain and culture
- Biochemical tests is required to identify the species

**Treatment**:

- third generation cephalosporins
- fluoroquinolones

**Prevention and control**:

- Although prevention of hospital acquired infections is difficult, meticulous hand washing best way to minimize transmission.

As yet, vaccines or hyperimmune sera are not available

## *Proteus vulgaris*

**Morphology**:

a. Gram negative rod, motile, non-spore forming, non-encapsulated, facultative anaerobic
b. Opportunistic pathogen
c. Reservoirs -Humans (normal flora of the GI tract), water, soil
d. Transmission - Fecal-oral, direct contact, contaminated water.

**Fig: *Proteus vulgaris*, a gram-negative bacterium is demonstrated by the red/pink colouration from the gram stain**

**Cultural Characteristics**:

a. The genus *Proteus* is classified in the **enteric bacteria**,
b. Gram-negative rods and are **facultative anaerobes**:
c. they ferment sugars in anaerobic conditions but can use a wide range of organic molecules in aerobic conditions.
d. The cells are highly motile and often **swarm** across the surface of agar plates. Swarming gives rise to a very thin film of bacteria on the agar surface, but swarming periods are interspersed with periods when the cells stop and undergo a cycle of growth and division so that the colony has a distinct zonation, clearly
e. It has the ability to degrade urea to ammonia, by production of the enzyme **urease**. This distinguishes them from the other enterics and is used in a simple diagnostic test.

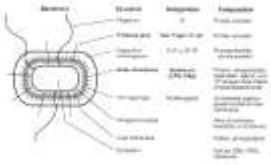
***Fig: Circular, smooth, entire, opaque colonies of Proteus vulgaris on a nutrient agar plate***

**Fig (A): *P.vulgaris* swarming phenomena on agar media**
**Fig (B): Showing degradation of urea by *P. vulgaris***

**Antigenic structure**:

a. Antigens possessed by these organisms include the H (flagellar), K (capsular) and O (somatic).

b. Those organisms that possess fimbriae (common pili) use these appendages for adhesion purposes



**Pathogenesis**:

**Adherence factors**

Fimbriae - facilitate adherence and thus enhance the capacity of the organism to produce disease

**Inflammatory response**

IL-6/IL-8 secretion - attachment to uroepithelial cells initiates' secretion apoptosis and epithelial cell desquamation pyelonephritis - Bacterial production of urease increases risk bacteremia & sepsis - bacterial endotoxin (LPS)

**Survival urease production** - alkalinize the urine by hydrolyzing urea to ammonia makes proteus effective in producing an environment in which it can survive.

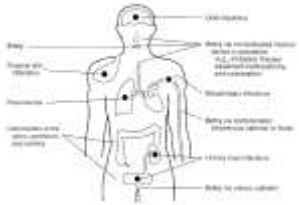


**Fig: Sites of colonization and extraintestinal disease production by *Proteus vulgaris***

**Clinical Features:**

Wound infections

- bronchopneumonia
- cystitis and urolithiasis
- septicemia

**Laboratory diagnosis**:

**Specimen**: Urine, pus, blood, ear discharge

**Smear**: Gram-negative rods

**Culture**: Produce characteristic swarming growth over the surface of blood agar.

Ditching of culture media prevents spread of proteus species. Non-lactose fermenting colonies in mac conkey agar.Proteus species have a characteristic smell.

**Biochemical reaction**:

Proteus spp…….. Urease positive

P. vulgaris……... Indole positive

**Serology:** Cross react with Weil-fellix test

**Treatment**:

- Ciprofloxacin
- Ceftazidime
- Netilmicin
- Sulbactam or Cefoperazo
- Meropenem
- Piperacillin/tazobactam
- Unasyn

Antibiotics should be introduced in much higher doses than "normal" when P. vulgaris has infected the sinus or respiratory tissues. I.E.- Ciprofloxacin should be introduced at a level of at least 2000 mg per day orally in such a situation, rather than the "standard" 1000 mg per day.

**Prevention and control**:

a. **Sanitary**: Frequent hand-washing by staff and a general awareness of microbial presence can reduce hospital-acquired infections. Disinfectants are not always effective.
b. **Immunological**: There is the possibility of anti-serum or vaccine against these organisms but none are currently in use.

**Chemotherapeutic**: Moderate or broad spectrum antibiotics are generally useful. Susceptibility tests should be performed when appropriate.

## *Pseudomonas aeruginosa*

**Morphology**:

a. Gram negative rod, encapsulated, motile, non-spore forming, obligate aerobic bacteria 0.5 to 0.8 µm by 1.5 to 3.0 µm.
b. opportunistic pathogen
c. Reservoirs - humans (normal flora of the GI tract), water, soil
d. Transmission - direct contact, droplet nuclei, contaminated water, contaimanted soil contaminated fomites

**Fig:Gram stain of *Pseudomonas aeruginosa* cells**

**Cultural Characteristics**:

a. Pseudomonads are Gram negative rods.
b. They are motile, nonfermentative aerobes
c. Can utilize acetate for carbon and ammonium sulphate for nitrogen.
d. Many species are resistant to high salt, dyes, weak antiseptics most antibiotics.
e. *P. aeruginosa* optimally at 37 °C, and it is able to grow at temperatures as high as at 42°C.

**Fig: *Pseudomonas aeruginosa* in cetrimide agar**

**Antigenic structure:**

- Exotoxin A
- Exoenzyme S

The cell envelope of *P aeruginosa*, which is similar to that of other Gram-negative bacteria, consists of three layers: the inner or cytoplasmic membrane, the peptidoglycan layer, and the outer membrane. The outer membrane is composed of phospholipid, protein, and lipopolysaccharide (LPS). The LPS of *P aeruginosa* is less toxic than that of other Gram-negative rods. The LPS of most strains of *P aeruginosa*

contains heptose, 2-keto-3-deoxyoctonic acid, and hydroxy fatty acids, in addition to side-chain and core polysaccharides. Recent evidence suggests that the LPS of a large percentage of strains isolated from patients with cystic fibrosis may have little or no polysaccharide side chain (O antigen), and that this finding correlates with the polyagglutinability of these strains with typing sera.

**athogenesis**

*P. aeruginosa* is infamously known for its ingenious mode of infection. Once this pathogen enters its host, it releases an exotoxin known as A-B toxin. This toxin is composed of two units, namely: A and B. Toxin B binds to the cell and is internalized; the A subunit, which is bound to the B subunit, has toxic activity and is also internalized . Exotoxin A then binds to a coenzyme inside the cell called *nicotinamide adenine dinucleotide* (NAD) ; NAD is involved in many cellular oxidation-reduction reactions. The binding of exotoxin releases the nicotinamide from *adenine dinucleotide* (ADP-ribose). This ADP-ribose unit stays attached to the A subunit. Exotoxin A transfers ADP-ribose to Elongation Factor-2, a protein involved in the translation and elongation of proteins . This, in turn, blocks protein synthesis in host cells, causing damaging to target tissues. Another way *P. aeruginosa* evades the immune system is by producing specialized microbial factors that inactivate the complement cascade. Recall that the complement system is a biochemical cascade that helps or 'complements' the ability of antibodies to clear pathogens from an organism in innate immunity. These enzymes known as *elastases* cleaves and destroys complement proteins, specifically C3a and C5a , resulting in a reduction in the local immune response to *P. aeruginosa* and favouring the establishment of infection .

***In Healthy***

- Endophthalmitis, keratitis and corneal ulcers (primarily if contact lenses)
- Otitis externa (“swimmer’s ear”)

***In Immunocompromized***

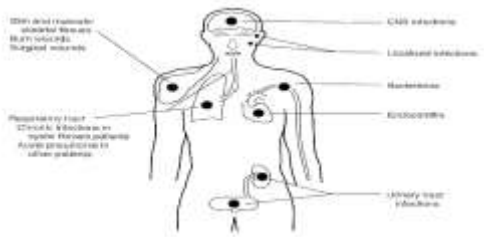
- Second most common cause of acute infectious endocarditis
- Wound infections (primarily if burned)
- Tracheobronchitis and bronchopneumonia (primarily if intubated)
- Chronic bronchopneumonia and severe progressive pulmonary abscesses (primarily if cystic fibrosis)
- Cystitis and pyelonephritis (primarily if catheterized)
- Meningitis (primarily if external ventricular drainage catheter) - septicemia

**Laboratory diagnosis**:

*P aeruginosa* depends on its isolation and laboratory identification. It grows well on most laboratory media and commonly is isolated on blood agar plates or eosin-methylthionine blue agar. It is identified on the basis of its Gram morphology, inability to ferment lactose, a positive oxidase reaction, its fruity odor, and its ability to grow at 4 2° C. Fluorescence under ultraviolet radiation helps in early identification of *P aeruginosa* colonies and also is useful in suggesting its presence in wounds. Other pseudomonads are identified by specific laboratory tests.

**Treatment** –

- Extended spectrum penicillins in conjunction with aminoglycosides (due to high antibiotic resistance)



**Prevention and control:**

The spread of *P aeruginosa* can best be controlled by observing proper isolation procedures, aseptic technique, and careful cleaning and monitoring of respirators, catheters, and other instruments. Topical therapy of burn wounds with antibacterial agents such as mafenide or silver sulfadiazine, coupled with surgical debridement, has dramatically reduced the incidence of *P aeruginosa* sepsis in burn patients.

*Pseudomonas aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed, making susceptibility testing essential. The combination of gentamicin and carbenicillin is frequently used to treat severe *Pseudomonas* infections, especially in patients with leukopenia. Several types of vaccines are being tested, but none is currently available for general use.

## *Vibrio cholerae*

**Morphology**:

a. Gram negative curved rod (comma shaped), motile, non-spore forming, non-encapsulated, facultative anaerobic, facultative alkaliphilic bacteria.
b. Obligate isotonic (may only survive in isotonic environments)
c. Obligate pathogen
d. Reservoirs - humans (not normal flora), water
e. Transmission - fecal-oral, contaminated water, contaminated food

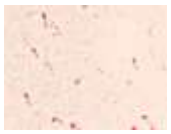


**Fig: Vibrio cholerae. Leifson flagella stain**

**Cultural Characteristics**:

- *V. cholerae* is also motile due to its polar flagellum and makes ATP (an energy molecule) by aerobic respiration if oxygen is present, but is also capable of switching to fermentation - *facultative anaerobe*.
- Species belonging to the genus *Vibrio* are distinguished from *Enterobacteria* by their flagella, as well as being oxidase positive.
- They grow in temperatures around 37°C and can survive in conditions as low as 25°C.
- *V. cholerae* generally require saltwater to grow properly, but a glucose medium is usually enough for their energy needs.

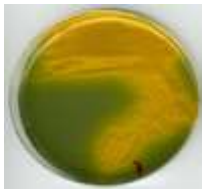


**Fig: *Vibrio cholerae* colonies in TCBS(Thiosulfate Citrate Bile Sucrose) agar**

**Antigenic structure**:

- Choleragen
- LPS

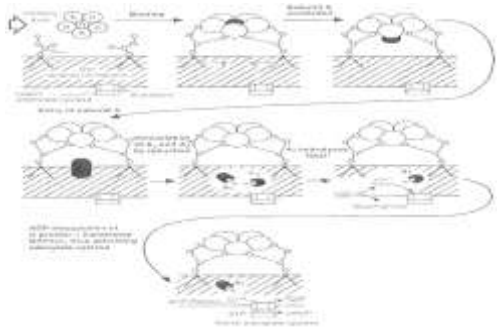


**Fig: Mechanism of action of cholera enterotoxin**

The enterotoxin works by activating adenylate cyclase in the epithelial cells. More specifically, it inhibits GTPase enzyme activity so that GDP (an inhibitory molecule) cannot replace the activated G protein which has GTP bound to it in its active state during signal transduction. The activated G protein goes on to activate adenylyl cyclase, producing large amounts of another chemical compound called cAMP. cAMP then activates protein kinase A, an enzyme that induces several different cellular responses, including the release of ions. cAMP also opens plasma membrane calcium channels, so that large amounts of calcium are released into the intestines, leading to an increase in water loss. Cholera toxin modifies the G protein by adding an ADP-ribosyl group to it. This prevents the enzyme GTPase, as mentioned earlier, from hydrolyzing the activated the G protein to a deactivated state which has GDP bound to it, as opposed to GTP. As a result, if the G protein is constantly active, there is persistent activation of adenylyl cyclase and, in turn, a nonregulated rise of intracellular cAMP.

**Pathogenesis**:

Cholera is transmitted by the fecal-oral route. Vibrios are sensitive to acid, and most die in the stomach. Surviving virulent organisms may adhere to and colonize the small bowel, where they secrete the potent cholera enterotoxin (CT, also called "choleragen"). This toxin binds to the plasma membrane of intestinal epithelial cells and releases an enzymatically active subunit that causes a rise in cyclic adenosine $5^1$-monophosphate (cAMP) production. The resulting high intracellular cAMP level causes massive secretion of electrolytes and water into the intestinal lumen.

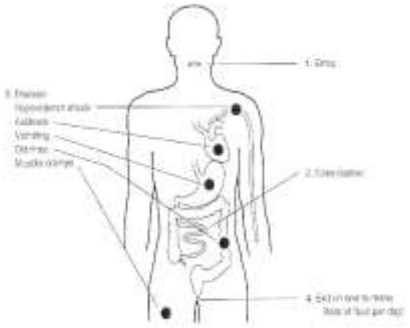


**Fig: Pathophysiology of cholera**

**Clinical Features:**

***Cholera Gravis***

- Severe watery diarrhea (>25 liter per day)
- May lead to hypovolemia " hypovolemic shock

- 50% mortality if untreated (within hours)
- Caused by Cholera Gravis infection of the GI tract " production and secretion of choleragen
- The syndrome of "cholera gravis" is characterized by "rice water" stool with volumes over 1L/hour leading to rapid dehydration and death.

**Laboratory diagnosis**:

The diagnosis is suggested by strikingly severe, watery diarrhea. For rapid diagnosis, a wet mount of liquid stool is examined microscopically. The characteristic motility of vibrios is stopped by specific antisomatic antibody. Other methods are culture of stool or rectal swab samples on TCBS agar and other selective and nonselective media; the slide agglutination test of colonies with specific antiserum; fermentation tests (oxidase positive); and enrichment in peptone broth followed by fluorescent antibody tests, culture, or retrospective serologic diagnosis. More recently the polymerase chain reaction (PCR) and additional genetically-based rapid techniques have been recommended for use in specialized laboratories.

**Treatment**:

Treatment of cholera consists essentially of replacing fluid and electrolytes. Formerly, this was accomplished intravenously, using costly sterile pyrogen-free intravenous solutions. The patient's fluid losses were conveniently measured by the use of buckets, graduated in half-liter volumes, kept underneath an appropriate hole in an army-type cot on which the patient was resting. Antibiotics such as tetracycline, to which the vibrios are generally sensitive, are useful adjuncts in treatment. They shorten the period of infection with the cholera vibrios, thus reducing the continuous source of cholera enterotoxin; this results in a substantial saving of replacement fluids and a markedly briefer hospitalization.

**Prevention and control**:

Control by sanitation is effective but not feasible in endemic areas.A good vaccine has not yet been developed. A parenteral vaccine of whole killed bacteria has been used widely, but is relatively ineffective and is not generally recommended. An experimental oral vaccine of killed whole cells and toxin B-subunit protein is less than ideal. Living attenuated genetically engineered mutants are promising, but such strains can cause limited diarrhea as a side effect. Antibiotic prophylaxis is feasible for small groups over short periods.

## *Salmonella enterica (typhi)*

**Morphology**:

- Gram negative rod, encapsulated, motile, non-spore forming, facultative anaerobic, facultative intracellular , facultative alkaliphilic bacteria.
- Obligate pathogen
- Reservoirs -Humans (only reservoir, not normal flora)
- Transmission - direct contact, fecal-oral, contaminated water, contaminated food

**Fig: *Salmonella typhi* gram negative rods**

**Cultural Characteristics**:

- Coliform bacilli (enteric rods)
- Motile gram-negative facultative anaerobes
- Non-lactose fermenting
- Resistant to bile salts
- H2S producing

**Antigenic structure**:

As with all *Enterobacteriaceae,* the genus *Salmonella* has three kinds of major antigens with diagnostic or identifying applications: somatic, surface, and flagellar.

**Somatic (O) or Cell Wall Antigens**

Somatic antigens are heat stable and alcohol resistant. Cross-absorption studies individualize a large number of antigenic factors, 67 of which are used for serological identification. O factors labeled with the same number are closely related, although not always antigenically identical.

**Surface (Envelope) Antigens**

Surface antigens, commonly observed in other genera of enteric bacteria (e.g., *Escherichia coli* and *Klebsiella),* may be found in some *Salmonella* serovars. Surface antigens in *Salmonella* may mask O antigens, and the bacteria will not be agglutinated with O antisera. One specific surface antigen is well known: the Vi antigen. The Vi antigen occurs in only three *Salmonella* serovars (out of about 2,200): Typhi, Paratyphi C, and Dublin. Strains of these three serovars may or may not have the Vi antigen.

**Flagellar (H) Antigens**

Flagellar antigens are heat-labile proteins. Mixing salmonella cells with flagella-specific antisera gives a characteristic pattern of agglutination (bacteria are loosely attached to each other by their flagella and can be dissociated by shaking). Also, antiflagellar antibodies can immobilize bacteria with corresponding H antigens.

**Pathogenesis**:

**Enteric Fevers**

***a.*** ***S. typhi*** causes **typhoid fever**
***S. paratyphi A, B*** (*S. schottmuelleri*) and ***C*** (*S. hirschfeldii*) cause milder form of enteric fever

b. **Infectious dose** = 106 CFU

c. **Fecal-oral route of transmission**
   i. Person-to-person spread by chronic carrier
   ii. Fecally-contaminated food or water

d. **10-14 days incubation period**

e. Initially signs of **sepsis/bacteremia** with **sustained fever** (delirium) for > one week before abdominal pain and **gastrointestinal symptoms**

**f. Invasiveness**

i. Pass through intestinal epithelial cells in ileocecal region, infect the regional lymphatic system, invade the bloodstream, and infect other parts of the reticuloendothelial system

ii. Organisms are phagocytosed by macrophages and monocytes, but survive, multiply and are transported to the liver, spleen, and bone marrow where they continue to replicate

iii. Second week: organisms reenter bloodstream and cause prolonged bacteremia; biliary tree and other organs are infected; gradually increasing sustained fever likely from endotoxemia

iv. Second to third week: bacteria colonize gallbladder, reinfect intestinal tract with diarrheal symptoms and possible necrosis of the Peyer's patches

**Clinical Features:**

***Typhoid Fever***

- "Enteric fever"
- Enterocolitis (high fever, headache, abdominal pain, vomiting and watery diarrhea) and mesenteric lymphadenitis ("mock appendicitis") " abdominal rash ("rose spots"), hepatosplenomegaly and generalized lymphadenomegaly

- caused by phagocytosis of Salmonella Typhi by macrophages of the gut-associated lymphoid tissue ("GALT") " survival of *Salmonella typhi* within the macrophages " dissemination of *Salmonella typhi* in virtually every lymphoid organ

**Laboratory diagnosis**:

The diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens. Laboratory identification of the genus *Salmonella* is done by biochemical tests; the serologic type is confirmed by serologic testing. Feces, blood, or other specimens should be plated on several nonselective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, *Salmonella-Shigella*, and brilliant green agars) as well as intoenrichment broth such as selenite or tetrathionate. Any growth in enrichment broth is subsequently subcultured onto the various agars. The biochemical reactions of suspicious colonies are then determined on triple sugar iron agar and lysine-iron agar, and a presumptive identification is made. Biochemical identification of salmonellae has been simplified by systems that permit the rapid testing of 10–20 different biochemical parameters simultaneously. The presumptive biochemical identification of *Salmonella* then can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera. Fortunately, approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. *Salmonella* isolates then should be sent to a central or reference laboratory for more comprehensive serologic testing and confirmation.

**Treatment**:

Enteric fever and bacteremia require antibiotic treatment: chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole. Surgical drainage of metastatic abscesses may be required

**Prevention and control**:

Effective vaccines exist for typhoid fever but not for non-typhoidal salmonellosis. Those diseases are controlled by hygienic slaughtering practices and thorough cooking and refrigeration of food.

a. **Antibiotics to avoid carrier state**
b. **Identify & treat carriers** of *S. typhi* & *S. paratyphi*
c. **Vaccination** can reduce risk of disease for **travellers in endemic areas**

## *Shigella dysenteriae*

**Morphology**:

- Gram negative rod, nnon-encapsulated, non-motile , non-spore forming ,facultative anaerobic intracellular
- Obligate pathogen
- Reservoirs -Humans (only reservoir, not normal flora)
- Transmission- direct contact,,fecal-oral, vectorial (flies), contaminated water, contaminated food.

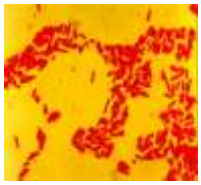


**Fig: *Shigella dysenteriae* cultured in Gram stain**

**Cultural Characteristics**:

- Coliform bacilli (enteric rods)
- Nonmotile gram-negative facultative anaerobes
- Does not ferment lactose
- Gas is not produced from glucose fermentation
- IMViC is -+--

- Does not produce lysine decarboxylase
- H2S and urease negative
- Four species
  i. *Shigella sonnei* (most common in industrial world)
  ii. *Shigella flexneri* (most common in developing countries)
  *iii. Shigella boydii*
  iv. *Shigella dysenteriae*
- Non-lactose fermenting
- Resistant to bile salts

**Fig: *Shigella dysenteriae* Non lactose fermenting colonies in Mac Conkey Agar plate**

**Antigenic structure**:
Shiga toxin causes hemorrhagic bacillary dysentery and hemolytic-uremic syndrome

- Enterotoxic, neurotoxic and cytotoxic
- Encoded by chromosomal genes
- Two domain (A-5B) structure
- Similar to the Shiga-like toxin of enterohemorrhagic E. coli (EHEC)

NOTE: except that Shiga-like toxin is encoded by lysogenic bacteriophage

**Pathogenesis**:

***Shigellosis***

**Two-stage disease:**

- **Early stage:**
  - **Watery diarrhea** attributed to the **enterotoxic activity of Shiga toxin** following ingestion and **noninvasive colonization**, multiplication, and production of enterotoxin in the **small intestine**
  - **Fever** attributed to **neurotoxic activity** of toxin
- **Second stage:**
  - Adherence to and tissue **invasion of large intestine** with typical symptoms of **dysentery**
  - **Cytotoxic activity** of Shiga toxin increases severity

**Enterotoxic Effect:**

- Adheres to **small intestine receptors**
- **Blocks absorption** (uptake) **of electrolytes, glucose, and amino acids** from the intestinal lumen

**Note**: This contrasts with the effects of **cholera toxin** (*Vibrio cholerae*) and labile toxin **(LT)** of enterotoxigenic *E. coli* (ETEC) which act by **blocking absorption of Na+**, but also cause **hypersecretion of water and ions** of Cl-, K+ (low potassium = hypokalemia), and HCO3- (loss of bicarbonate buffering capacity leads to metabolic acidosis) out of the intestine and into the lumen

**Cytotoxic Effect**:

- B subunit of Shiga toxin binds host cell glycolipid
- A domain is internalized via receptor-mediated endocytosis (coated pits)
- Causes irreversible inactivation of the 60S ribosomal subunit, thereby causing:

- Inhibition of protein synthesis
- Cell death
- Microvasculature damage to the intestine
- Hemorrhage (blood & fecal leukocytes in stool)

**Neurotoxic Effect**: Fever, abdominal cramping are considered signs of neurotoxicity

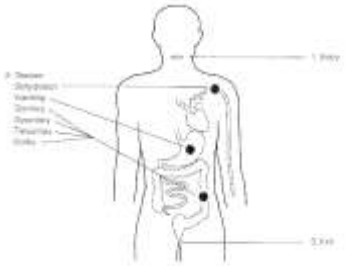


**Fig: Pathogenesis of shigellosis in humans**

**Clinical Features:**

***Bacillary Dysentery***

- low-grade fever, abdominal cramps, abdominal pain, vomiting and purulent hemorrhagic diarrheaspontaneously resolves in < 1 week
- primarily occurs in children and elderly
- analogous to hemorrhagic colitis
- caused by Shigella infection of the GI tract " production and secretion of shiga toxin " necrosis of the enterocytes
- Some cases were accompanied by hemolytic uremic syndrome (HUS).

**Laboratory diagnosis**:

- **Specimens**: fresh stool, mucus flecks, and rectal swabs. Large numbers of fecal leukocytes and some RBC may often be seen microscopically.
- **Culture**: differential and selective media as used for salmonellae.

**Treatment**:

- broad spectrum penicillins in conjunction with oral fluid and electrolyte replacement (if bacillary dysentery)
- broad spectrum penicillins in conjunction with careful oral fluid and electrolyte replacement (if hemolytic-uremic syndrome, due to occlusion of the glomeruli)

**Prevention and control**:

Humans are the only reservoir for shigellae.

- Transmission of shigellae: water, food, fingers, feces, and flies.
- Most cases occur in children under 10 years of age.
- Prevention and control of dysentery:
- Sanitary control of water, food and milk; sewage disposal; and fly control.
- Isolation of patients and disinfection of excreta.
- Detection of subclinical cases and carriers.

## *Treponema pallidum*

**Morphology**:

a. Gram negative spirochete (helically-coiled cell), motile, non-spore forming, non-encapsulated microaerophilic bacteria, 6 to 15 μm long and 0.1 to 0.2 μm wide.

b. obligate intracellular pathogen
c. Reservoirs -Humans (only reservoir, not normal flora)
d. Transmission - direct contact, sexual, perinatal
e. Too thin to be seen with light microscopy in specimens stained with Gram stain or Giemsa stain
f. Motile spirochetes can be seen with **darkfield micoscopy**
g. Staining with **anti-treponemal antibodies labeled with fluorescent dyes**

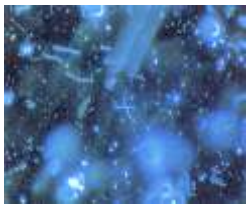


**Fig: Darkfield Microscopy of *Treponema pallidum***

**Cultural Characteristics**:

a. The *Treponema* are motile, helically coiled organisms having a corkscrew-like shape,.
b. They stain very poorly because their thickness approaches the resolution of the light microscope.
c. *Treponema* are delicate organisms requiring pH in the range 7.2 to 7.4, temperatures in the range 30°C to 37°C and a microaerophilic environment.
d. The structure of these organisms is somewhat different: the cells have a coating of glycosamino-glycans, which may be host-derived, and the outer membrane covers the three flagella that provide motility.
e. In addition, the cells have a high lipid content (cardiolipin, cholesterol), which is unusual for most bacteria. Cardiolipin elicits "Wassermann" antibodies that are diagnostic for syphilis.
f. Multiplication is by binary transverse fission.
g. Treponemes have not yet been cultured in vitro.

**Antigenic structure**:

- Outer membrane proteins promote adherence
- Hyaluronidase may facilitate perivascular infiltration
- Antiphagocytic coating of fibronectin
- Tissue destruction and lesions are primarily result of host's immune response (immunopathology)

**Pathogenesis**:

Humans are the only natural host for *T pallidum* subsp *pallidum*, and infection occurs through sexual contact. The organisms penetrate mucous membranes or enter minuscule breaks in the skin. In women the initial lesion is usually on the labia, the walls of the vagina, or the cervix; in men it is on the shaft or glans of the penis. A chancre also may occur on lips, tongue, tonsils, anus, or other skin areas. The observation, made in a number of in vitro studies, that *T pallidum* subsp *pallidum* and subsp *pertenue* specifically attach to numerous cell types is believed to reflect the ability of these bacteria to infect diverse tissues and organs. To disseminate away from the site of initial entry, organisms must traverse the viscous ground substance between tissue cells. There is evidence that *Treponema pallidum* subsp *pallidum* elaborates an enzyme capable of degrading hyaluronic acid within the ground substance, thereby potentially facilitating hematogenous dissemination of organisms.

**Clinical Features:**

***Primary Syphilis***

- a single small painless depressed ulcer with elevated margins ("chancre") at the site of initial infection, fever, headache, anorexia and local lymphadenomegaly

- primarily occurs on the external genitalia, periorally (if oral intercourse) or perianally (if anal intercourse)
- occurs 3-6 weeks after initial infection
- spontaneously resolves in 3-6 weeks
- caused by *Treponema pallidum* infection of the skin
- may progress to secondary syphilis

***Secondary Syphilis***

- small flat erythematous rashes of the palms and soles, small painless papules ("condyloma lata") of the groin and axilla, and generalized lymphadenomegaly
- occurs 12-18 weeks after initial infection
- spontaneously resolves in 3-6 weeks
- caused by *Treponema pallidum* septicemia
- may progress to tertiary syphilis

***Tertiary Syphilis***

- nodular well circumscribed caseating granulomas ("gummas") of the skin, liver and bone, obliterative endarteritis of the vasa vasorum leading to aortic aneurysm ("cardiovascular syphilis"), meningitis, obliterative endarteritis of the cerebral arteries leading to cerebral infarct, and permanent central neuronal damage leading to general paresis and tabes dorsalis ("neurosyphilis")
- occurs 3-15 years after initial infection
- occurs in 30% of untreated patients
- caused by *Treponema pallidum* accumulation in tissues

***Early Congenital Syphilis***

- small flat erythematous rashes of the palms and soles, condylomalata of the groin and axilla, osteitis, rhinitis (snuffles"), hepatosplenomegaly and generalized lymphadenomegaly
- occurs immediately after birth
- spontaneously resolves in 1-3 weeks
- caused by intrauterine *Treponema pallidum* infection "

***Treponema pallidum* septicemia**

- may progress to late congenital syphilis

***Late Congenital Syphilis***

- gummas (see above) of the cartilage of the nose, the bone of the hard palate and the teeth leading to deformation of the face ("bulldog facies"), gummas of the tibia and fibula leading to deformation of the legs ("saber shins"), and neurosyphilis
- occurs 1-3 years after birth
- caused by *Treponema pallidum* accumulation in tissues

**Laboratory diagnosis**:

**Tests:** After the examination, a blood test for syphilis will be done. Besides this you may be tested for other sexually transmitted infections, including HIV.

**Blood test:**

Infection with syphilis causes your body to make antibodies against the syphilis bacteria. When your blood is tested, it may show the antibodies that are present. A positive result means that you either have the infection or have had it in the past (the antibodies persist in the body for years; even after successfully treatment of an infection). If the blood test is negative, it does not necessarily mean that you are not

infected. The antibodies against syphilis bacteria may not be detected for up to three months after infection. Your doctor may advise repeat test after three months to confirm the diagnosis. Blood test for syphilis is done in every pregnant woman as the infection can affect the baby (it can result in death of the foetus or newborn baby or cause other complications). The test is usually done at weeks 11-20 of pregnancy. The common blood tests done for syphilis include:

- RPR (rapid plasma reagin).
- VDRL (venereal disease research laboratory).
- FTA-ABS (fluorescent treponemal antibody absorption) or MHA-TP (microhemagglutination assay for T pallidum).

**Swab:** If the patient has sores the doctor will take a sample from the sore and examine it under a microscope (perform a dark-field microscope examination). The test is useful in the primary and also sometimes in the secondary phase of infection.

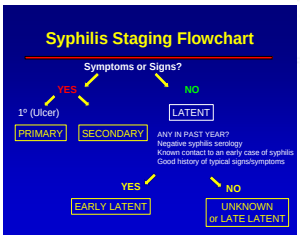
**Spinal fluid examination:** During the tertiary phase, the examination of a sample of spinal fluid obtained by spinal puncture may be done to check for infection and to measure the success of treatment.

**Treatment**:

a. Recommended regimen:
   i. Benzathine penicillin G 2.4 million units IM once
b. Non-pregnant penicillin-allergic adults *
c. Data to support the use of alternatives to penicillin are limited and if used, close follow-up is essential
   i. Doxycycline 100mg orally twice daily for two weeks or
   j. Tetracycline 500mg orally 4 times a day for two weeks or
   k. Adherance is poor (i.e., dosing and gastrointestinal effects)
   l. Ceftriaxone1 g IM daily x 8-10 d or
   m. (Azithromycin 2 g po)…not recommended in CA
d. Efficacy in HIV + persons not studied so use with caution

**Prevention and control**:

a. Sanitary: As with other sexually transmitted diseases (STDs), use of a condom helps prevent infection.
b. Immunological: None are available.
c. Chemotherapeutic: Benzathine penicillin (long acting) or penicillin G are the drugs of choice. One must be aware of a possible Jarisch-Herxheimer reaction following treatment of secondary or tertiary syphilis, however. The rapid release of treponemal antigens after lysis by penicillin can cause hypersensitivity reactions in some persons.



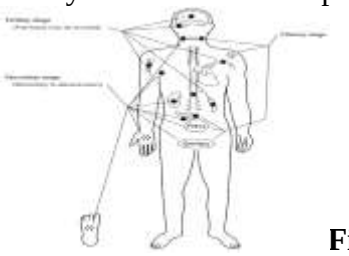



**Fig: Clinical manifestations of syphilis**

## *Leptospira Sp.,*

Leptospirosis is a zoonosis of worldwide distribution. It is caused by spirochetes of the genus *Leptospira*. The traditional classification system is based on biochemical and serologic specificity to differentiate between the pathogenic species, *Leptospira* interrogans, and the free-living nonpathogenic species,

*Leptospira biflexa*. The species are further broken down to more than 200 serovars of *L interrogans* and more than 60 serovars of *L biflexa*. The serovars are further organized into serogroups of *L interrogans* and serogroups of *L biflexa*. The serogroups are based on shared antigenicity and are primarily for laboratory use. A second classification system is based on DNA–DNA hybridization studies, which have demonstrated a high degree of heterogeneity within the two species of the traditional classification. Phylogenetic analysis based on 16S rRNA gene sequencing indicates that there are three clades of leptospires, pathogens, saprophytes, and some of uncertain pathogenicity. The 19 species (13 pathogenic and six saprophytic) do not correspond to the species in the traditional serologic classification. Indeed, some serovars in the traditional classification occur in multiple species in the molecular classification, and the serologic classification cannot be used to predict the molecular classification. Under the new classification, the species are further subdivided into 24 serogroups and 250 serovars based on the surface lipopolysaccharide.

*Leptospira* (Greek *leptos*, "fine, thin" and Latin *spira*, "coil") is a genus of spirochaete bacteria, including a small number of pathogenic and saprophytic species. *Leptospira* was first observed in 1907 in kidney tissue slices of a leptospirosis victim who was described as having died of "yellow fever."

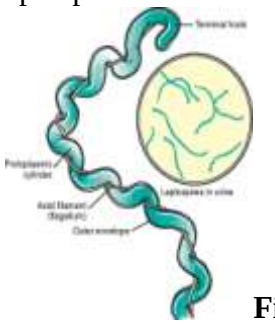


**Fig: Morphology of *Leptospira***

**Morphology**

- Spiral-shaped bacteria
- Delicate, flexible, helical rods
- Actively motile and hooked ends
- 6-20 μm long and 0.1 μm in diameter with a wavelength of about 0.5 μm.
- They are so thin, live *Leptospira* are best observed by darkfield microscopy.

**Fig: *Leptospira* under dark field microscope**

**Cultural characteristics**

- Temperature:
  - Optimum temperature range: 28-30°C; extreme range: 11-42°C
  - Growth at 11-13°Chas been proposed as a phenotypic test for *L.biflexa*
  - Pathogens grows in mammalian host at febrile body temperature; in chick embryos and young chicks at 40-42°C
- pH range: 6.5-8.4
- Growth:
  - It is an obligate anaerobe

- Leptospires grow best under aerobic conditions at 28–30°C in semisolid medium (eg, Ellinghausen-McCullough-Johnson- Harris EMJH) in 10 mL test tubes with 0.1% agar and 5-fluorouracil
- Leptospira strain grow slowly, Colonies can take from 3-7 days to 3 weeks to appear
- Routinely grow in liquid media at 30°C with a doubling time of 6-8h under optimum condition
- Leptonema grows rapidly in medium reach maximum density on incubation for 18-72h at 30°C

- Growth Requirements:
  Leptospirae derive energy from oxidation of long-chain fatty acids and cannot use amino acids or carbohydrates as major energy sources. Ammonium salts are a main source of nitrogen. Leptospirae can survive for weeks in water, particularly at alkaline pH.

**Antigenic Structure:**

The main strains ("serovars") of *L interrogans* isolated from humans or animals in different parts of the world are all serologically related and exhibit crossreactivity in serologic tests. This indicates considerable overlapping in antigenic structure, and quantitative tests and antibody absorption studies are necessary for a specific serologic diagnosis. The outer envelope contains large amounts of lipopolysaccharide of antigenic structure that is variable from one strain to another. This variation forms the basis for the serologic classification of the *Leptospira* species. It also determines the specificity of the human immune response to leptospirae.

**Principal Leptospiral Diseases**

| *Leptospira* Serogroups | Source of Infection | Disease in Humans | Clinical Findings | Distribution |
|---|---|---|---|---|
| Autumnalis | ? | Pretibial fever or Ft. Bragg fever | Fever, rash over tibia | United States, Japan |
| Ballum | Mice | — | Fever, rash, jaundice | United States, Europe, Israel |
| Bovis | Cattle, voles | — | Fever, prostration | United States, Israel, Australia |
| Canicola | Dog urine | Infectious jaundice | Influenza-like illness, aseptic meningitis | Worldwide |
| Grippotyphosa | Rodents, water | Marsh fever | Fever, prostration, aseptic meningitis | Europe, United States, Africa |
| Hebdomadis | Rats, mice | 7-day fever | Fever, jaundice | Japan, Europe |
| Icterohaemorrhagiae | Rat urine, water | Weil disease | Jaundice, hemorrhages, aseptic meningitis | Worldwide |
| Mitis | Swine | Swineherd's disease | Aseptic meningitis | Australia |
| Pomona | Swine, cattle | Swineherd's disease | Fever, prostration, aseptic meningitis | Europe, United States, Australia |

**Pathogenesis and Clinical Findings**

Human infection usually results from leptospires, often in bodies of water, entering the body through breaks in the skin (cuts and abrasions) and mucous membranes (mouth, nose, conjunctivae). Ingestion is considered to be less important. After an incubation period of 1–2 weeks, there is a variable febrile onset during which spirochetes are present in the bloodstream. They then establish themselves in the parenchymatous organs (particularly liver and kidneys), producing hemorrhage and necrosis of tissue and resulting in dysfunction of those organs (jaundice, hemorrhage, nitrogen retention). The illness is often biphasic. After initial improvement, the second phase develops when the IgM antibody titer rises. It manifests itself often as "aseptic meningitis" with an intense headache, stiff neck, and pleocytosis of the CSF. Nephritis and hepatitis may also recur, and there may be skin, muscle, and eye lesions. The degree and distribution of organ involvement vary in the different diseases produced by different leptospirae in

various parts of the world (see Table 24-1). Many infections are mild or subclinical. Hepatitis is frequent in patients with leptospirosis.

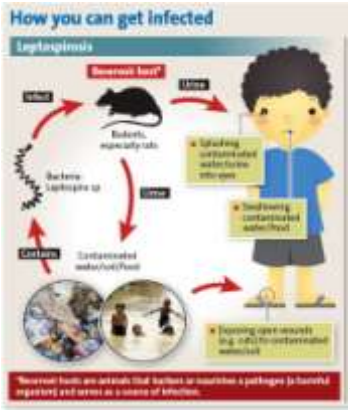



**Fig: Mode of Transmission**

Kidney involvement in many animal species is chronic and results in the shedding of large numbers of leptospirae in the urine; this is probably the main source of environmental contamination resulting in infection of humans. Human urine also may contain spirochetes in the second and third weeks of disease. Agglutinating, complement-fixing, and lytic antibodies develop during the infection. Serum from convalescent patients protects experimental animals against an otherwise fatal infection. The immunity resulting from infection in humans and animals appears to be serovar specific.

**Diagnostic Laboratory Tests**

**A. Specimens**

Specimens consist of aseptically collected blood in a heparin tube, CSF, or tissues for microscopic examination and culture. Urine should be collected using great care to avoid contamination. Serum is collected for agglutination tests.

**B. Microscopic Examination**

Dark-field examination or thick smears stained by the Giemsa technique occasionally show leptospirae in fresh blood from early infections. Results of dark-field examination of centrifuged urine may also be positive. Fluorescein-conjugated antibodies or other immunohistochemical techniques can be used also.

**C. Culture**

Whole fresh blood or urine can be cultured in a semisolid medium. Because of inhibitory substances in blood, only 1 or 2 drops should be placed in each of five tubes containing 5 or 10 mL of medium. Up to 0.5 mL of CSF can be used. One drop of undiluted urine can be used followed by 1 drop each of 10-fold serially diluted urine for a total of four tubes. Tissue approximately 5 mm in diameter should be crushed and used as the inoculum. Growth is slow, and cultures should be kept for at least 8 weeks.

**D. Serology**

The diagnosis of leptospirosis in most cases is confirmed serologically. Agglutinating antibodies first appear 5–7 days after infection and develop slowly, reaching a peak at 5–8 weeks. Very high titers may be attained (>1:10,000). The reference laboratory standard for detection of leptospiral antibody uses microscopic agglutination of live organisms, which can be hazardous. The test is highly sensitive, but it is difficult to standardize; the end point is 50% agglutination, which is difficult to determine. Agglutination of the live suspensions is most specific for the serovar of the infecting leptospires. Agglutination tests are generally performed only in reference laboratories. Paired sera that show a significant change in titer or a single serum with high-titer agglutinins plus a compatible clinical illness can be diagnostic. Because of the difficulty in performing the definitive agglutination tests, a variety of other tests have been developed for use primarily as screening tests.

**Immunity**

Serovar-specific immunity follows infection, but reinfection with different serovars may occur.

**Treatment**

Treatment of mild leptospirosis should be with oral doxycycline, ampicillin, or amoxicillin. Treatment of moderate or severe disease should be with intravenous penicillin or ampicillin.

**Epidemiology, Prevention, and Control**

The leptospiroses are essentially animal infections; human infection is only accidental, occurring after contact with water or other materials contaminated with the excreta of animal hosts. Rats, mice, wild rodents, dogs, swine, and cattle are the principal sources of human infection. They excrete leptospirae in urine both during the active illness and during the asymptomatic carrier state. Leptospirae remain viable in stagnant water for several weeks; drinking, swimming, bathing, or food contamination may lead to human infection. Persons most likely to come in contact with water contaminated by rats (eg, miners, sewer workers, farmers, and fishermen) run the greatest risk of infection. Children acquire the infection from dogs more frequently than adults do. Control consists of preventing exposure to potentially contaminated water and reducing contamination by rodent control. Doxycycline, 200 mg orally once weekly during heavy exposure, is effective prophylaxis. Dogs can receive distemper–hepatitis–leptospirosis vaccinations.

## *Neiserria Sp.,*

The neisseriae are gram-negative cocci that usually occur in pairs (diplococci). *Neisseria gonorrhoeae* (gonococci) and *Neisseria meningitidis* (meningococci) are pathogenic for humans and typically are found associated with or inside polymorphonuclear cells. Some neisseriae are normal inhabitants of the human respiratory tract, rarely if ever cause disease, and occur extracellularly.

Gonococci and meningococci are closely related, with 70% DNA homology, and are diff erentiated by a few laboratory tests and specific characteristics: Meningococci have polysaccharide capsules but gonococci do not, and meningococci rarely have plasmids but most gonococci do. Most importantly, the two species are diff erentiated bythe usual clinical presentations of the diseases they cause: Meningococci typically are found in the upper respiratory tract and cause meningitis, but gonococci cause genital infections. The clinical spectra of the diseases caused by gonococci and meningococci overlap, however.

**Morphology :**

**A. Typical Organisms**

The typical *Neisseria* is a gram-negative, nonmotile diplococcus, approximately 0.8 μm in diameter. Individual cocci are kidney shaped; when the organisms occur in pairs, the fl at or concave sides are adjacent.

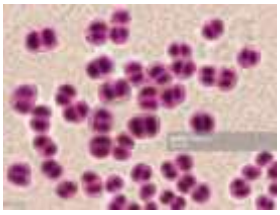


**Fig: Grams staing**

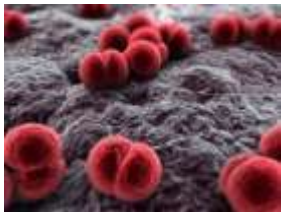


**Fig: Electron microscopy**

**B. Culture**

In 48 hours on enriched media (eg, modified Thayer-Martin, Martin-Lewis, GC-Lect, and New York City), gonococci and meningococci form convex, glistening, elevated, mucoid colonies 1–5 mm in diameter. Colonies are transparent or opaque, nonpigmented, and nonhemolytic. *Neisseria fl avescens, Neisseria cinerea, Neisseria subfl ava,* and *Neisseria lactamica* may have yellow pigmentation. *Neisseria sicca* produces opaque, brittle, wrinkled colonies. *Moraxella catarrhalis* produces nonpigmented or pinkish gray opaque colonies.

**C. Growth Characteristics**

The neisseriae grow best under aerobic conditions, but some grow in an anaerobic environment. They have complex growth requirements. Most neisseriae oxidize carbohydrates, producing acid but not gas, and their carbohydrate patterns are a means of distinguishing them (see Table 20-1 ). The neisseriae produce oxidase and give positive oxidase reactions; the oxidase test is a key test for identifying them. When bacteria are spotted on a filter paper soaked with tetramethylparaphenylenediamine hydrochloride (oxidase), the neisseriae rapidly turn dark purple.

Meningococci and gonococci grow best on media containing complex organic substances such as heated blood, hemin, and animal proteins and in an atmosphere containing 5% CO 2 (eg, candle jar). Growth is inhibited by some toxic constituents of the medium (eg, fatty acids or salts). The organisms are rapidly killed by drying, sunlight, moist heat, and many disinfectants. Th ey produce autolytic enzymes that result in rapid swelling and lysis in vitro at 25°C and at an alkaline pH.

**Antigenic Heterogeneity of *Neisseria gonorrhoeae***

| Antigen | Number of Types |
|---|---|
| Pilin | Hundreds |
| Por (protein) (U.S. system) | PorA with 18 subtypes PorB with 28 subtypes |
| Opa (protein II) | Many (perhaps hundreds) |
| Rmp (protein III) | One |
| Lipooligosaccharide | Eight or more |
| Fbp (iron-binding protein) | One |
| Lip (H8) | One |
| IgA1 protease | Two |

**Pathogenesis, Pathology, and Clinical Findings**

Gonococci exhibit several morphologic types of colonies (see earlier discussion), but only piliated bacteria appear to be virulent. Opa protein expression varies depending on the type of infection. Gonococci that form opaque colonies are isolated from men with symptomatic urethritis and from uterine cervical cultures at midcycle. Gonococci that form transparent colonies are frequently isolated from men with asymptomatic urethral infection; from menstruating women; and from patients with invasive forms of gonorrhea, including salpingitis and disseminated infection. Antigenic variation of surface proteins during infection allows the organism to circumvent host immune response.

Gonococci attack mucous membranes of the genitourinary tract, eye, rectum, and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis. Men usually have urethritis, with yellow, creamy pus and painful urination.

The process may extend to the epididymis. As suppuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures. Urethral infection in men can be asymptomatic. In women, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. It may then progress to the uterine tubes, causing salpingitis, fibrosis, and obliteration of the tubes. Infertility occurs in 20% of women with gonococcal salpingitis. Chronic gonococcal cervicitis and proctitis are often asymptomatic.

Gonococcal bacteremia leads to skin lesions (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists. Gonococci can be cultured from blood or joint fluid of only 30% of patients with gonococcal arthritis. Gonococcal endocarditis is an uncommon but severe infection. Gonococci sometimes cause meningitis and eye infections in adults; these have manifestations similar to those caused by meningococci.

Complement deficiency is frequently found in patients with gonococcal bacteremia. Patients with bacteremia, especially if recurrent, should be tested for total hemolytic complement activity. Gonococcal ophthalmia neonatorum, an infection of the eye in newborns, is acquired during passage through an infected birth canal. The initial conjunctivitis rapidly progresses and, if untreated, results in blindness. To prevent gonococcal ophthalmia neonatorum, instillation of tetracycline, erythromycin, or silver nitrate into the conjunctival sac of newborns is compulsory in the United States. Gonococci that produce localized infection are often serum sensitive (ie, killed by antibody and complement).

**Diagnostic Laboratory Tests**

**A. Specimens**

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear. Blood culture is necessary in systemic illness, but a special culture system is helpful because gonococci (and meningococci) may be susceptible to the polyanethol sulfonate present in standard blood culture media.

**B. Smears**

Gram-stained smears of urethral or endocervical exudates reveal many diplococci within pus cells. These give a presumptive diagnosis. Stained smears of the urethral exudates from men have a sensitivity of about 90% and a specificity of 99%. Stained smears of endocervical exudates have a sensitivity of about 50% and a specificity of about 95% when examined by an experienced microscopist. Additional diagnostic testing of urethral exudates from men is not necessary when the stain result is positive, but nucleic acid amplification tests (NAATs) or cultures should be done for women. Stained smears of conjunctival exudates can also be diagnostic, but those of specimens from the throat or rectum are generally not helpful.

**C. Culture**

Immediately after collection, pus or mucus is streaked on enriched selective medium (eg, modified Thayer-Martin medium [MTM]) and incubated in an atmosphere containing 5% CO2 (candle extinction jar) at 37°C. To avoid overgrowth by contaminants, the selective medium contains antimicrobial drugs (eg, vancomycin, 3 μg/mL; colistin, 7.5 μg/mL; amphotericin B, 1 μg/mL; and trimethoprim, 3 μg/mL). If immediate incubation is not possible, the specimen should be placed in a CO2-containing transport-culture system. Fortyeight hours after culture, the organisms can be quickly identifiedby their appearance on a Gram-stained smear; by oxidase positivity; and by coagglutination, immunofluorescence staining, or other laboratory tests. The species of subcultured bacteria may be determined by oxidation of specific carbohydrates (see Table 20-1). Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has potential to provide rapid (same-day) identification of cultured isolates. The gonococcal isolates from anatomic sites other than the genital tract or from children should be identified as to species using two different confirmatory tests because of the legal and social implications of a positive culture result.

**D. Nucleic Acid Amplification Tests**

Several Food and Drug Administration–cleared nucleic acid amplification assays are available for direct detection f *N gonorrhoeae* in genitourinary specimens, and these are the preferred tests from these sources. In general, these assays have excellent sensitivity and specificity in symptomatic, high-prevalence populations. Advantages include better detection, more rapid results, and the ability to use urine as a specimen source. Disadvantages include poor specificity of some assays because of cross-reactivity with nongonococcal *Neisseria* species. These assays are not recommended for use for the diagnosis of extragenital gonococcal infections or for infection in children. NAATs are not recommended as tests of cure because nucleic acid may persist in patient specimens for up to 3 weeks after successful treatment.

**E. Serology**

Serum and genital fluid contain immunoglobulin G (IgG) and IgA antibodies against gonococcal pili, outer membrane proteins, and LPS. Some IgM of human sera is bactericidal for gonococci in vitro. In infected individuals, antibodies to gonococcal pili and outer membrane proteins can be detected by immunoblotting, radioimmunoassay, and ELISA (enzyme-linked immunosorbent assay) tests. However, these tests are not useful as diagnostic aids for several reasons, including gonococcal antigenic heterogeneity, the delay in development of antibodies in acute infection, and a high background level of antibodies in the sexually active population.

**Immunity**

Repeated gonococcal infections are common. Protective immunity to reinfection does not appear to develop as part of the disease process, because of the antigenic variety of gonococci. Although antibodies can be demonstrated, including the IgA and IgG on mucosal surfaces, they are either highly strain specific or have little protective ability.

**Treatment**

Since the development and widespread use of penicillin, gonococcal resistance to penicillin has gradually risen, owing to the selection of chromosomal mutants, so that many strains now require high concentrations of penicillin G for inhibition (MIC ≥2 μg/mL). Penicillinase-producing *N gonorrhoeae* (PPNG) also have increased in prevalence. Chromosomally mediated resistance to tetracycline (MIC ≥2 μg/mL) is common. High-level resistance to tetracycline (MIC ≥32 μg/mL) also occurs. Spectinomycin resistance as well as resistance to fluoroquinolones has been noted. Single-dose fluoroquinolone treatment was recommended for treatment of gonococcal infections from 1993 until 2006. Since 2006, rates of quinolone resistance among gonococcal isolates have exceeded 5% in men who have sex with men and in heterosexual men. Because of the problems with antimicrobial resistance in *N gonorrhoeae*, the Centers for Disease Control and Prevention (CDC) recommends that patients with uncomplicated genital or rectal infections be treated with ceftriaxone (250 mg) given intramuscularly as a single dose or 400 mg of oral cefixime as a single dose. Additional therapy with 1 g of azithromycin orally in a single dose or with 100 mg of doxycycline orally twice a day for 7 days is recommended for possible concomitant chlamydial infections. Azithromycin has been found to be safe and effective in pregnant women, but doxycycline is contraindicated. Modifications of these therapies are recommended for other types of *N gonorrhoeae* infection. See the CDC's website for the 2010 updated treatment guidelines. Because other sexually transmitted diseases may have been acquired at the same time as gonorrhea, steps must also be taken to diagnose and treat these diseases (see discussions of chlamydiae, syphilis, and so on).

**Epidemiology, Prevention, and Control**

Gonorrhea is worldwide in distribution. In the United States, its incidence rose steadily from 1955 until the late 1970s, when the incidence was between 400 and 500 cases per 100,000 populations. Between 1975 and 1997, there was a 74% decline in the rate of reported gonococcal infections. Thereafter, the rates plateaued for 10 years, decreased from 2006–2009, but increased by 2.8% between 2009 and 2010. Gonorrhea is exclusively transmitted by sexual contact, often by women and men with asymptomatic infections. The infectivity of the organism is such that the chance of acquiring infection from a single exposure to an infected sexual partner is 20–30% for men and even greater for women. The infection rate can be reduced by avoiding multiple sexual partners, rapidly eradicating gonococci from infected individuals by means of early diagnosis and treatment, and finding cases and contacts through education and screening of populations at high risk. Mechanical prophylaxis (condoms) provides partial protection. Chemoprophylaxis is of limited value because of the rise in antibiotic resistance of the gonococcus.

Gonococcal ophthalmia neonatorum is prevented by local application of 0.5% erythromycin ophthalmic ointment or 1% tetracycline ointment to the conjunctiva of newborns. Although instillation of silver nitrate solution is also effective and is the classic method for preventing ophthalmia neonatorum, silver nitrate is

difficult to store and causes conjunctival irritation; its use has largely been replaced by use of erythromycin or tetracycline ointment.

## *Haemophilus Sp.,*

This is a group of small, gram-negative, pleomorphic bacteria that require enriched media, usually containing blood or its derivatives, for isolation. *Haemophilus infl uenzae* type b is an important human pathogen; *Haemophilus ducreyi*, a sexually transmitted pathogen, causes chancroid; other *Haemophilus* species are among the normal microbiota of mucous membranes and only occasionally cause disease.

### *Haemophilus influenza*

*Haemophilus infl uenzae* is found on the mucous membranes of the upper respiratory tract in humans. It is an important cause of meningitis in children and causes upper and lower respiratory tract infections in children and adults.

**Morphology and Identifi cation**

**A. Typical Organisms**

In specimens from acute infections, the organisms are short (1.5 μm) coccoid bacilli, sometimes occurring in pairs or short chains. In cultures, the morphology depends both on the length of incubation and on the medium. At 6–8 hours in rich medium, the small coccobacillary forms predominate. Later there are longer rods, lysed bacteria, and very pleomorphic forms. Organisms in young cultures (6–18 hours) on enriched medium have a defi nite capsule. Th e capsule is the antigen used for "typing" *H infl uenzae*.

**B. Culture**

On chocolate agar, fl at, grayish brown colonies with diameters of 1–2 mm are present aft er 24 hours of incubation. IsoVitaleX in media enhances growth. *H infl uenzae* does not grow on sheep blood agar except around colonies of staphylococci ("satellite phenomenon"). *Haemophilus haemolyticus* and *Haemophilus parahaemolyticus* are hemolytic variants of *H infl uenzae* and *Haemophilus parainfluenzae*, respectively.

**C. Growth Characteristics**

Identifi cation of organisms of the *Haemophilus* group depends partly on demonstrating the need for certain growth factors called X and V. Factor X acts physiologically as hemin; factor V can be replaced by nicotinamide adenine nucleotide (NAD) or other coenzymes. Colonies of staphylococci on sheep blood agar cause the release of NAD, yielding the satellite growth phenomenon. Carbohydrates are fermented poorly and irregularly. In addition to serotyping on the basis of capsular polysaccharides, *H infl uenzae* and *H parainfluenzae* can be biotyped on the basis of the production of indole, ornithine decarboxylase and urease. Most of the invasive infections caused by *H influenzae* belong to biotypes I and II (there are a total of eight).

**D. Variation**

In addition to morphologic variation, *H infl uenzae* has a marked tendency to lose its capsule and the associated type specificity. Nonencapsulated variant colonies lack iridescence.

**E. Transformation**

Under proper experimental circumstances, the DNA extracted from a given type of *H influenzae* is capable of transferring that type specifi city to other cells (transformation). Resistance to ampicillin and chloramphenicol is controlled by genes on transmissible plasmids.

**Antigenic Structure**

Encapsulated *H infl uenzae* contains **capsular polysaccharidesm** (molecular weight >150,000) of one of six types (a–f). The capsular antigen of type b is a polyribitol ribose phosphate (PRP). Encapsulated *H infl uenzae* can be typed by slide agglutination, coagglutination with staphylococci, or agglutination of latex particles coated with type-specifi c antibodies. A capsule swelling test with specific antiserum is analogous to the quellung test for pneumococci. Typing can also be done by immunofluorescence. Most *H influenzae* organisms in the normal microbiota of the upper respiratory tract are not encapsulated. The somatic antigens of *H influenzae* consist of outer membrane proteins. Lipooligosaccharides (endotoxins) share many structures with those of neisseriae.

**Pathogenesis**

*H influenzae* produces no exotoxin. The nonencapsulated organism is a regular member of the normal respiratory microbiota of humans. The capsule is antiphagocytic in the absence of specific anticapsular antibodies. The polyribose phosphate capsule of type b *H influenzae* is the major virulence factor. The carrier rate in the upper respiratory tract for *H influenzae* type b was 2–4% in the prevaccine era and is now less than 1%. The carrier rate for nontypeable *H influenza* is 50–80% or higher. Type b *H influenzae* causes meningitis, pneumonia and empyema, epiglottitis, cellulitis, septic arthritis, and occasionally other forms of invasive infection. Nontypeable *H influenzae* tends to cause chronic bronchitis, otitis media, sinusitis, and conjunctivitis after breakdown of normal host defense mechanisms. The carrier rate for the encapsulated types a and c to f is low (1–2%), and these capsular types rarely cause disease. Although type b can cause chronic bronchitis, otitis media, sinusitis, and conjunctivitis, it does so much less commonly than nontypeable *H influenzae.* Similarly, nontypeable *H influenzae* only occasionally causes invasive disease (~5% of cases). The blood of many persons older than age 3–5 years is bactericidal for *H influenzae*, and clinical infections are less frequent in such individuals. However, bactericidal antibodies have been absent from 25% of adults in the United States, and clinical infections have occurred in adults.

**Clinical Findings**

*H influenzae* type b enters by way of the respiratory tract. There may be local extension with involvement of the sinuses or the middle ear. *H influenzae,* mostly nontypeable, and pneumococci are two of the most common etiologic agents of bacterial otitis media and acute sinusitis. Encapsulated organisms may reach the bloodstream and be carried to the meninges or, less frequently, may establish themselves in the joints to produce septic arthritis. Before the use of the conjugate vaccine, *H influenzae* type b was the most common cause of bacterial meningitis in children age 5 months to 5 years in the United States. Clinically, it resembles other forms of childhood meningitis, and diagnosis rests on bacteriologic demonstration of the organism. Occasionally, a fulminating obstructive laryngotracheitis with swollen, cherry-red epiglottis develops in infants and requires prompt tracheostomy or intubation as a lifesaving procedure. Pneumonitis and epiglottitis caused by *H influenza* may follow upper respiratory tract infections in small children and old or debilitated people. Adults may have bronchitis or pneumonia caused by *H influenzae.*

**Diagnostic Laboratory Tests**

**A. Specimens**

Specimens consist of expectorated sputum and other types of respiratory specimens, pus, blood, and spinal fluid for smears and cultures depending on the source of the infection.

**B. Direct Identification**

Commercial kits are available for immunologic detection of *H influenzae* antigens in spinal fluid. A positive test result indicates that the fluid contains high concentrations of specific polysaccharide from *H influenzae* type b. These antigen detection tests generally are not more sensitive than a Gram stain and therefore are not widely used, especially because the incidence of *H influenzae* meningitis is so low.

**C. Culture**

Specimens are grown on IsoVitaleX-enriched chocolate agar until typical colonies appear. *H influenzae* is differentiated from related gram-negative bacilli by its requirements for X and V factors and by its lack of hemolysis on blood agar. Tests for X (heme) and V (nicotinamide-adenine dinucleotide) factor requirements can be done in several ways. The *Haemophilus* species that require V factor grow around paper strips or disks containing V factor placed on the surface of agar that has been autoclaved before the blood was added(V factor is heat labile). Alternatively, a strip containing X factor can be placed in parallel with one containing V factor on agar deficient in these nutrients. Growth of *Haemophilus* in the area between the strips indicates requirement for both factors. A better test for X factor requirement is based on the inability of *H influenzae* (and a few other *Haemophilus* species) to synthesize heme from δ-aminolevulinic acid. The inoculum is incubated with the δ-aminolevulinic acid. *Haemophilus* organisms that do not require X factor synthesize porphobilinogen, porphyrins, protoporphyrin IX, and heme. The presence of red fluorescence under ultraviolet light (~360 nm) indicates the presence of porphyrins and a positive test result. *Haemophilus* species that synthesize porphyrins (and thus heme) are not *H influenza*.

**Immunity**

Infants younger than age 3 months may have serum antibodies transmitted from their mothers. During this time, *H influenzae* infection is rare, but subsequently, the antibodies are lost. Children often acquire *H influenzae* infections, which are usually asymptomatic but may be in the form of respiratory disease or meningitis. *H influenzae* was the most common cause of bacterial meningitis in children from 5 months to 5 years of age until the early 1990s when the conjugate vaccines became available. By age 3–5 years, many unimmunized children have naturally acquired anti-PRP antibodies that promote complementdependent bactericidal killing and phagocytosis. Immunization of children with *H influenzae* type b conjugate vaccine induces the same antibodies. There is a correlation between the presence of bactericidal antibodies and resistance to major *H influenzae* type b infections. However, it is not known whether these antibodies alone account for immunity. Pneumonia or arthritis caused by infection with *H influenzae* can develop in adults with such antibodies.

**Treatment**

The mortality rate for individuals with untreated *H influenza* meningitis may be up to 90%. Many strains of *H influenza* type b are susceptible to ampicillin, but up to 25% produce β-lactamase under control of a transmissible plasmid and are resistant. Essentially all strains are susceptible to the thirdgeneration cephalosporins. Cefotaxime given intravenously gives excellent results. Prompt diagnosis and antimicrobial therapy are essential to minimize late neurologic and intellectual impairment. Prominent among late complications of *H influenzae* type b meningitis is the development of a localized subdural accumulation of fluid that requires surgical drainage.

**Epidemiology, Prevention, and Control**

Encapsulated *H influenzae* type b is transmitted from person to person by the respiratory route. *H influenzae* type b disease can be prevented by administration of ***Haemophilus* b conjugate vaccine** to children. Currently, two conjugate vaccines are available for use: PRP-OMPC (polysaccharide linked to outer membrane protein complex), the outer membrane protein complex of *Neisseria meningitidis* serogroup B, and PRP-T, which uses tetanus toxoid. Beginning at age 2 months, all children should be immunized with one of the conjugate vaccines. Depending on which vaccine product is chosen, the series consists of three doses at 2, 4, and 6 months of age or two doses given at 2 and 4 months of age. An additional booster dose is given sometime between 12 and 15 months of age. Both conjugate vaccines can be given at the time of other vaccine administration such as DTaP (diphtheria, tetanus, and acellular pertussis). Widespread use of *H influenza* type b vaccine has reduced the incidence of *H influenza* type b meningitis in children by more than 95%. The vaccine reduces the carrier rates for *H influenzae* type b. Contact with patients with *H influenzae* type b clinical infection poses little risk for adults but presents a

definite risk for nonimmune siblings and other nonimmune children younger than age 4 years who are close contacts. Prophylaxis with rifampin is recommended for such children.

## Possible Questions

### Part B (Two marks)

1. Write about the morphology of *E.coli*?
2. Comment on the biochemical property of *E.coli*.
3. Give the morphology of *Klebsiella*.
4. Comment on the biochemical property of *Klebsiella*.
5. Write about the morphology of *Proteus?*
6. Comment on the biochemical property of *Proteus*.
7. Discuss the cultural characteristics of *Klebsiella*.
8. Give the morphology of *Pseudomonas*.
9. Write about the morphology of *Vibrio*?
10. Give the morphology of *Salmonella*.
11. . Comment on the biochemical property of *Salmonella.*
12. Write about the morphology of *Shigella*?
13. Give the morphology of *Treponema*.
14. . Comment on the biochemical property of *Treponema.*
15. Write about Laboratory diagnosis of *Proteus*?
16. Give the morphology of *Haemophilus*.
17. Discuss the cultural characteristics of *Pseudomonas*.

### Part C (Eight marks)

1. Discuss the cultural characteristics of *E.coli.*
2. Discuss the antigenic property of *E.coli*.
3. Write about Laboratory diagnosis of *E.coli*?
4. Comment on the biochemical property of *Pseudomonas*.
5. Explain the antigenic property of *Pseudomonas.*
6. Outline the Laboratory diagnosis of *Pseudomonas*.
7. Explain the antigenic property of *Klebsiella.*
8. Outline the Laboratory diagnosis of *Klebsiella*.
9. Comment on the biochemical property of *Vibrio*.
10. Discuss the cultural characteristics of *Vibrio*.
11. Discuss the antigenic property of *Vibrio*.
12. Write about Laboratory diagnosis of *Vibrio*?
13. Discuss the cultural characteristics of *Proteus*.
14. Discuss the cultural characteristics of *Salmonella*
15. Discuss the antigenic property of *Proteus.*
16. Explain the antigenic property of *Salmonella.*
17. Outline the Laboratory diagnosis of *Salmonella.*
18. Comment on the biochemical property of *Shigella*.
19. Discuss the cultural characteristics of *Shigella*.
20. Discuss the antigenic property of *Shigella*.
21. Write about Laboratory diagnosis of *Shigella*?
22. Discuss the cultural characteristics of *Treponema.*
23. Explain the antigenic property of *Treponema.*
24. Outline the Laboratory diagnosis of *Treponema.*
25. Write about the morphology of *Neisseria*?
26. Comment on the biochemical property of *Neisseria*.
27. Discuss the cultural characteristics of *Neisseria*.
28. Discuss the antigenic property of *Neisseria*.

29. Write about Laboratory diagnosis of *Neisseria?*
30. Discuss the cultural characteristics of *Haemophilus.*
31. Comment on the biochemical property of *Haemophilus*
32. Explain the antigenic property of *Haemophilus.*
33. Outline the Laboratory diagnosis of *Haemophilus*

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS: I M.Sc MB** **COURSE NAME: MEDICAL BACTERIOLOGY**

**COURSE CODE: 19MBP202** **UNIT: IV** **BATCH-2019-2021**

| S.No | Unit IV | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | Vibrio cholerae shows result for indole and citrate test. | negative | positive | neutral | no reaction | positive |
| 2 | CLED stands for _____agar . | cystine lactose electrolyte deficient | crystalviolet lactose electrolyte deficient | citrate lactose electrolyte deficient | cultural lactose electrolyte deficient | cystine lactose electrolyte deficient |
| 3 | Strains of V.cholerae O1 is subdivided into Inaba and _____ subtypes. | V.cholerae O2 | Hikojima | panama | Ogawa | Ogawa |
| 4 | Widal test is performed for the serological diagnosis of _____. | *V.cholerae O2* | *Salmonella* | *Klebsiella* | *Pseudomonas* | *Salmonella* |
| 5 | *E.coli* is an _____ in humans | Pathogen | predator | Parasite | commensal | Parasite |
| 6 | *E.coli* exhibits IMViC_____ | .++-- | --++ | .+-+- | ++++ | .++-- |
| 7 | The K antigen in *E.coli* is composed of _____ | Protein | lipid | | carbohydrate | Polysaccharide |
| 8 | ._____is known as travelers diarrhea | EPEC | ETEC | EHEC | EAEC | ETEC |
| 9 | EHEC is also known as_____ | EPEC | EAEC | VTEC | EXEC | VTEC |
| 10 | SIDS is seen in case of _____ | *E.coli* | *Klebsiella* | *Proteus* | *Pseudomonas* | *E.coli* |
| 11 | *Klebsiella* is_____ | Non motile and non capsulated | motile and capsulated | Non motile and capsulated | motile and non capsulated | Non motile and capsulated |
| 12 | *Klebsiella* exhibits_____ colonies | Dry | mucoid | Pale | diffuse | mucoid |
| 13 | *K. pneumoniae* is also known as _____ | Jansen's bacilli | Koch's bacilli | Friedlander's bacilli | Escherich's bacilli | Friedlander's bacilli |
| 14 | *Klebsiella* exhibits IMViC_____ | ++-- | --++ | +++ | ---- | --++ |
| 15 | The tribe proteae are_____ | Fermentors | . non fermentors | Late fermentors | early fermentors | . non fermentors |
| 16 | The proteae is classified into_____genera. | One | two | three | Four | three |
| 17 | Proteus exhibits _____ motility. | Swarming | no | Fish in stream | Darting | Swarming |
| 18 | The predominant aerobic bacterial flora of the large intestines of human beings and animals is | Non sporing bacilli | non acid fast bacilli | viruses | Gram negative | Gram negative bacilli |

| | | | | | | |
|---|---|---|---|---|---|---|
| | composed of_____ | | | | bacilli | |
| 19 | The clinical picture of dysentery is exhibited by_____ | *Mycobacteria* | *Pseudomonas* | *Klebsiella* | *Shigella* | *Shigella* |
| 20 | *Shigella* is _____ | Flagellated. | sporing | capsulated | Non motile | Non motile |
| 21 | The selective medium used for *Shigella* is_____ | Deoxycholate citrate agar | EMB | MSA | Martin Thayer | Deoxycholate citrate agar |
| 22 | The *Shigella* culture filtrates demonstrates_____ type toxicity. | Hypersensitive | Lytic | Chemotoxicity | Neurotoxicity | Neurotoxicity |
| 23 | The minimum infective dose for outcome of Shigellosis is_____ | 10-50 bacilli | 10-100 bacilli | 100-1000 bacilli | 1-10 bacilli | 10-100 bacilli |
| 24 | Bacillary dysentery has an incubation period of _____ | 6 hours | 1 day | 1-7 days | more than 7 days | 1-7 days |
| 25 | The main features of bacillary dysentery are_____ | Rice watery diarrhoea | presence of parasite | Abdominal discomfort | Loose scanty feces | Loose scanty feces |
| 26 | The infection with *Salmonella* is characterized by_____ | Malaise | gastric ulcer | Septicemia | Enteric fever | Enteric fever |
| 27 | *Salmonella* is known as_____bacilli. | Eberth gaffky | Shiga | Friedlander's | Escherich | Eberth gaffky |
| 28 | *Salmonella typhi* is the causative agent of _____ | Typhoid fever | paratyphoid fever | Yellow fever | Malaise | Typhoid fever |
| 29 | The incubation period of *Salmonella typhi* is_____ | 6 hours | 1 day | 1-7 days | 7-14 days | 7-14 days |
| 30 | The infective dose for *Salmonella typhi* is_____ | 1-10 bacilli | 100000 bacilli | 100000000 bacilli | 10000 bacilli | 100000000 bacilli |
| 31 | *Vibrio* is _____rods. | Helical | Elongated | Twisted | Curved | Curved |
| 32 | *Vibrio cholerae* was first isolated by_____ | Pasteur | Koch | Paccini | Boyd | Koch |
| 33 | *Vibrio* is_____ | Motile | Non motile | Slime | Capsulated. | Motile |
| 34 | *Vibrio cholerae* are_____rods. | Gram positive | Non motile | Spore forming. | curved, Cylindrical. | curved, Cylindrical. |
| 35 | ._____Is used as transport medium for Vibrio. | Alcohol medium | Acid | sea water | V R Medium | V R Medium |
| 36 | *Vibrio* colonies may be easily identified | Biochemical test | String test | Cultural | Coombs test | String test |

| | | | | | | |
|---|---|---|---|---|---|---|
| | by_____ | | | characteristics | | |
| 37 | Heiberg classified Vibrios into_____groups based on sugar fermentation. | 2 | 4 | 6 | 8 | 6 |
| 38 | The route of infection with Vibrio is by_____ route. | Oral | respiratory tract | Ingestion | inhalation | Oral |
| 39 | *Pseudomonas* is motile by_____flagella. | polar | bipolar | peritrichous | Atrichous | polar |
| 40 | *Vibrio is motile by_____ flagella.* | Atrichous | peritrichous | polar | Lopotrichous. | polar |
| 41 | Glycocalyx is composed of _____ | Protein | lipid | Polysaccharide | carbohydrate | Polysaccharide |
| 42 | *Pseudomonas produces*_____ *pigments.* | Ruby | lucosin | Pyocynanin | Verdin | Pyocynanin |
| 43 | Pyocyanin is a _____ colored pigment. | Blue | green | . Red | yellow | Blue |
| 44 | Pyocynanin is soluble in_____ | Acid | Base | chloroform , Water | ether | chloroform , Water |
| 45 | Fluorescin is_____ colored pigment. | Greenish yellow | Green | red | blue | Greenish yellow |
| 46 | Pyorubin is_____colored pigment | yellow | Green | red | blue | red |
| 47 | Pyomelanin is_____ colored pigment. | brown | red | Green | yellow | brown |
| 48 | *Pseudomonas aeruginosa* produces_____pigment | Pyocyanin | melanin | rubin | verdin | Pyocyanin |
| 49 | The term 'blue pus' is associated with_____ | *Proteus* | *Pseudomonas* | *E.coli* | *Vibrio* | *Pseudomonas* |
| 50 | ._____is used as selective media for Pseudomonas. | Citrimide agar | EMB | DCA | MSA | Citrimide agar |
| 51 | enteric fever is caused by _____. | Salmonella typhii | S. paratyphi D | S. enterica | Proteus | Salmonella typhii |
| 52 | EHEC strains are able to secrete _____. | verotoxin | exotoxin | endotoxin | exfoliate toxin | verotoxin |
| 53 | ._____isolated influenza bacillus. | Andrews and Laidlaw | Koch | Boyd | Pasteur | Andrews and Laidlaw |
| 54 | The accessory factors required by H. influenza is called as_____ | X and Y | X and V | X and Z | Y and Z | X and V |
| 55 | _____ enzyme aids the invasion of Pseudomonas into tissues | pectinase | elastase | protease | amylase | elastase |

| | | | | | | |
|---|---|---|---|---|---|---|
| 56 | ._____is the drug of choice for *Pseudomonas*. | Chloramiphenicol | Bacitracin | ceftazidime, Cefatoxime | Streptomycin | Chloramiphenicol |
| 57 | selective media for Pseudomonas aerugonisa is _____. | Mac conkey agar | blood agar | PLET | Dettol agar | Dettol agar |
| 58 | . *T. pallidum* causes_____ | Syphilis | typhoid | Tuberculosis | pertusis | Syphilis |
| 59 | *T. pallidum* are highly sensitive to _____ | Acid | Antiseptics | drying | antibiotic | drying |
| 60 | *Pseudomonas* mainly causes_____ | Primary infection | secondary infection | re- infection | nosocomial infection | nosocomial infection |

## UNIT-V
## SYLLABUS

Nosocomial infection – Urinary tract infection, Respiratory tract infection, Sexually transmitted disease – Immunoprophylaxis – Antimicrobial chemotheraphy, Antibiotics, second line drugs.Vaccines.

**Nosocomial infections:**

Nosocomial infections are infections are acquired in hospitals and other healthcare facilities. To be classified as a nosocomial infection, the patient must have been admitted for reasons other than the infection. He or she must also have shown no signs of active or incubating infection.

These infections occur:

- up to 48 hours after hospital admission
- up to 3 days after discharge
- up to 30 days after an operation
- in a healthcare facility when a patient was admitted for reasons other than the infection

In the United States, it has been estimated that 9.2 out of every 100 patients acquire a nosocomial infection

Nosocomial infections are caused by pathogens that easily spread through the body. Many hospital patients have compromised immune systems, so they are less able to fight off infections. In some cases, patients develop infections due to poor conditions at a hospital or a healthcare facility, or due to hospital staff not following proper procedures.

Some patients acquire nosocomial infections by interacting with other patients. Others encounter bacteria, fungi, parasites, or viruses in their hospital environment.

Symptoms of nosocomial infections vary by type. They include inflammation, discharge, fever, and abscesses. Patients may experience pain and irritation at the infection site, and many experience visible symptoms.

1. Urinary tract infections
2. Respiratory tract infections

**Urinary tract infections:**

A urinary tract infection (UTI) is an infection that affects part of the urinary tract. When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract it is known askidney infection (pyelonephritis). Symptoms from a lower urinary tract include pain with urination, frequent urination, and feeling the need to urinate despite having an empty bladder. Symptoms of a kidney infection includefever and flank pain usually in addition to the symptoms of a lower UTI. Rarely the urine may appear bloody. In the very old and the very young, symptoms may be vague or non-specific.

The most common cause of infection is Escherichia coli, though other bacteria or fungi may rarely be the cause. Risk factors include female anatomy, sexual intercourse, diabetes, obesity, and family history. Although sexual intercourse is a risk factor, UTIs are not classified as sexually transmitted infections (STIs). Kidney infection, if it occurs, usually follows a bladder infection but may also result from a blood-borne infection. Diagnosis in young healthy women can be based on symptoms alone. In those with vague symptoms, diagnosis can be difficult because bacteria may be present without there being an infection. In complicated cases or if treatment fails, a urine culture may be useful.

**Respiratory tract infection:**

Respiratory tract infection refers to any of a number of infectious diseasesinvolving the respiratory tract. An infection of this type is normally further classified as an upper respiratory tract infection (URI or

URTI) or a lower respiratory tract infection (LRI or LRTI). Lower respiratory infections, such aspneumonia, tend to be far more serious conditions than upper respiratory infections, such as the common cold.

**Upper respiratory tract infection**

Although some disagreement exists on the exact boundary between the upper and lower respiratory tracts, the upper respiratory tract is generally considered to be the airway above the glottis or vocal cords. This includes the nose,sinuses, pharynx, and larynx. Typical infections of the upper respiratory tract include tonsillitis, pharyngitis, laryngitis, sinusitis, otitis media, certain types of influenza, and the common cold. Symptoms of URIs can include cough, sore throat, runny nose, nasal congestion,headache, low grade fever, facial pressure and sneezing.

**Lower respiratory tract infection**

The lower respiratory tract consists of the trachea (wind pipe), bronchial tubes, the bronchioles, and the lungs.

Lower respiratory tract infections are generally more serious than upper respiratory infections. LRIs are the leading cause of death among all infectious diseases. The two most common LRIs are bronchitis and pneumonia. Influenza affects both the upper and lower respiratory tracts, but more dangerous strains such as the highly pernicious H5N1 tend to bind to receptors deep in the lungs.

**Immunoprophylaxis**

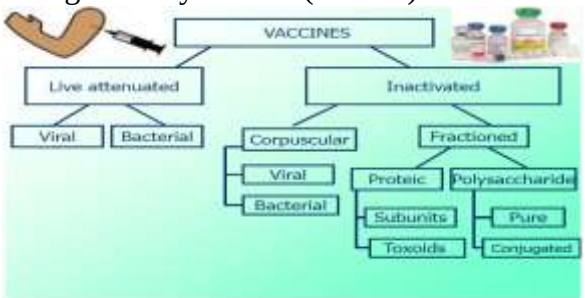
Immunoprophylaxis is an antiepidemic measure (of public health) taken in order to prevent the spreading of infectious diseases via immunization of the susceptible groups of the population.

**Main targets**

- Reducing the incidence (VHB, pertussis, mumps, rubella)
- Liquidation of the morbidity (eliminating the disease from certain territories –diphtheria, polio, measles)
- Eradication of the disease (smallpox)

**Discovery of Vaccination (Edward Jenner, 1796)**

- Edward Jenner (1749-1823) was a country doctor inGloucestershire county in the West of England. Heobserved that people who get cowpox often developless severe disease and survive smallpox outbreaks.
- Jenner inoculated a young boy (James Phipps) with material from hand sores of a milkmaid Sarah Nelmes. Six weeks later, after the boy recovered from cowpox, he was re-inoculated with the smallpox virus. – The boy survived …
- The term "Vaccination" was introduced by Jenner (from the Latin *Vacca,* and *Vaccinia* virus) and later adopted for immunization against any disease (Pasteur).




**Immunization**

Types of Immunizations

*1. **Active*** – exposure to antigen with the host generating protective immunity.

- Objective: provide long lasting immunity against futureExposures

*2.* ***Passive*** – administration of humoral and/or cellular factors that provide immunity for the host.

- Objective: provide temporary immediate protection against an imminent or ongoing exposure/threat

- "Herd" immunity in preventing spread of infection
- occurs when the vaccination of a significant portion of apopulation (or "herd") provides a measure of protection for individuals who have not developed immunity.

**Active Immunization**

2 Historical active vaccine approaches:

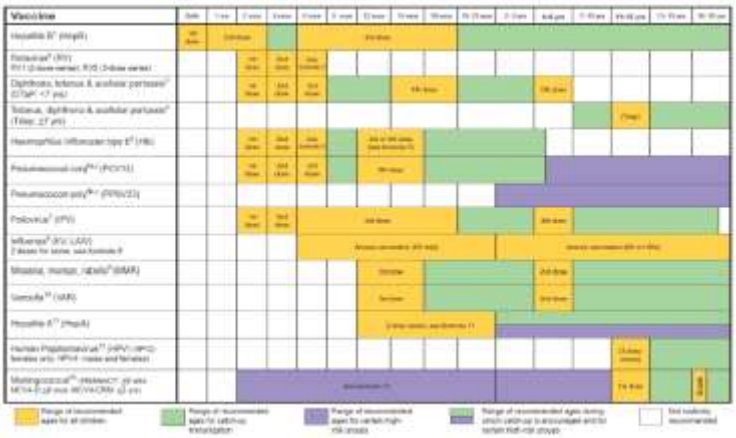
- **Attenuated** pathogen – ex. Rabies vaccine
- **Killed** pathogens – ex. Anthrax vaccine

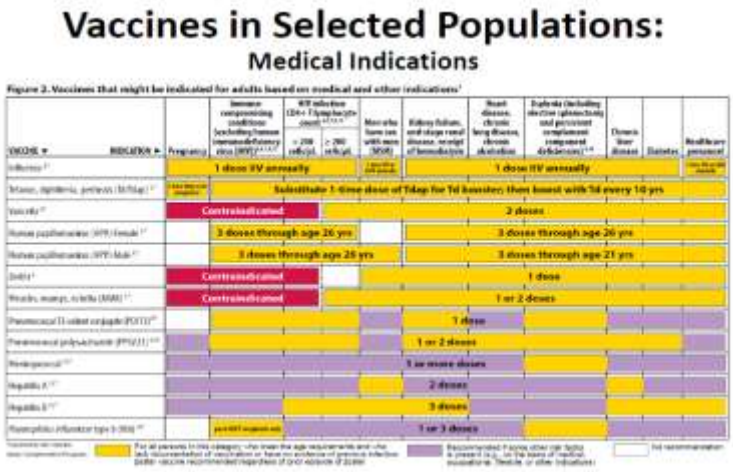
**Age and Timing of Immunizations**

**Children** under 2 yr are limited in producing antibodies to bacterial capsular polysaccharides.

- Limited response to T-independent antigens
- Can be overcome partially by chemical link to carrier protein
- Can assist maturation of response using multiple vaccinations

Recommended Immunization Schedule for persons age 0-18 years





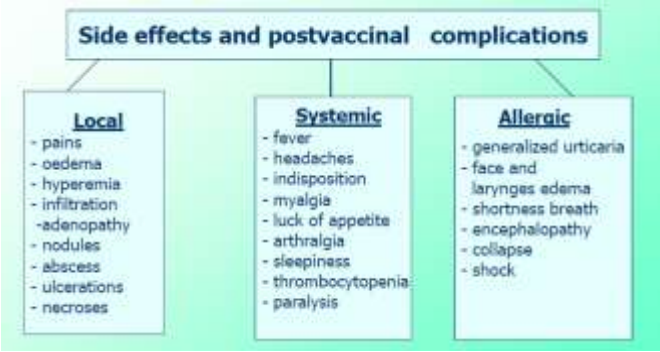

**General characteristic of the vaccines used for the immunoprophylaxis of infectious diseases included in the schedule**

- Viral hepatitis B – inactivated vaccine, proteic fractioned (HBsAg), plasmatic, biotechnologic
- *Tuberculosis – live attenuated vaccine, BCG*

- *Diphteria, Tetanus Pertusis – associated vaccine DTP, DTPa, DT, Td*
- *Polio – live attenuated vaccine (OPV), Corpuscular inactivated vaccine (IPV)*
- Measles, *Mumps, Rubella – live attenuated vaccine (MMR)*
- Haemophilus influenza type b (Hib) – inactivated vaccine, fractioned polysaccharide

**The role of immunoprophylaxis**

- Immunoprophylaxis of over 30 infectious diseases:
  smallpox, diphtheria, tetanus, pertussis, polio, measles, mumps, chikenpox, Haemophilus influenzae type b infection, VHA, VHB, TB, meningococcic infection, pneumococcic infection, influenza, typhoid fever, cholera, rabies, tick encephalitis, anthrax, yellow fever, rotaviral infection, tularemia etc.
- Infectious diseases in the prevention of which immunoprophylaxis holds the main role are called vaccine preventable diseases.
- Immunoprophylaxis as an individual protection: rabies, yellow fever etc.




**Measure of reducing the frequency of side effects and postvaccinal complications**

- selection of the persons for the vaccination
- observance of the immunization rules
- precocius registration and medical assistance
- evidence, epidemiological investigation and case analysis
- population information

**Antimicrobial Chemotheraphy:**

Microorganisms can grow on and within other organisms which leads to the development of disease, disability and death of an organism. Thus the control or destruction of microorganisms residing within the bodies of humans and other animals is of great importance. Modern medicine is dependent on **chemotherapeutic agents**, chemical agents that are used to treat diseases. Most of them are **antibiotics**, microbial products or their derivatives that can kill susceptible microbes or inhibit their growth.

The development of chemotherapy began with the work of German physician Paul Ehrlich, who reasoned that a chemical with selective toxicity that would kill pathogens and not human cells might be effective in treating disease. In 1904 he found that the dye trypan red was active against the trypanosome that causes African sleeping sickness and could be used therapeutically. Arsphenamine was active against syphilis. Dogmagk discovered that Protonoid red, a dye for staining leather, was nontoxic for animals and completely protected mice against pathogenic streptococci and staphylococci. Dogmagk has actually discovered sulphonamides or sulfa drugs and for this discovery he received Nobel Prize in 1939. The first antibiotic to be used therapeutically was penicillin. Stephan Fleming was the first to discover penicillin

from the fungus *Penicillium notatum*. Laterother scientists, Flare and Chain carried out the work further and the three scientists were awarded with Nobel Prize in 1945 for the discovery and production of penicillin. After the discovery of Penicillin, scientists became interested in other compounds produced by microbes which could be used as antibiotics. Selman Waksman announced in 1944 that he and his associates has found a new antibiotic, streptomycin, produced by the actinomycete *Streptomyces griseus* . He received the Nobel Prize in 1952 and his success led to a worldwide search for other antibiotic-producing microbes. Microorganisms producing chloramphenicol, neomycin, Terramycin and tetracycline were isolated by 1953. This led to the discovery of powerful drugs and has transformed modern medicine and greatly alleviated human suffering.

**General characteristics and mechanisms of action of antimicrobial agents**

A successful chemotherapeutic agent must have selective toxicity. The degree of selective toxicity may be expressed in terms of 1) the therapeutic dose, the drug level required for treatment of a particular infection and 2) the toxic dose, the drug level at which the agent becomes too toxic for the host. The therapeutic index is the ratio of the toxic dose to the therapeutic dose. The larger the TI, the better the chemotherapeutic agent. A drug that disrupts a microbial function not found in eukaryotic animal cells has a greater selective toxicity and a higher therapeutic index. Example, therapeutic index of penicillin is high as the action of penicillin is that it inhibits the peptidoglycan synthesis of bacterial cell walls, and as eukaryotic cells do not contain cell walls, the effect is minimum. The drug which has a low therapeutic index can cause undesirable effects on the host, called as side effects as the drug may inhibit the same process in host cells or damages the host in other ways. Hence, chemotherapeutic agents must be administered with great care.

Drugs can range in their effectiveness. Many are narrow-spectrum drugs- that are; they are effective only against a limited variety of pathogens, while the broad-spectrum drugs attack many different kinds of pathogens. Drugs can be classified according to the microbial group they act against; antibacterial, antifungal, antiprotozoan and antiviral. Chemotherapeutic agents can be synthesized by microorganisms or manufactured by chemical procedures independent of microbial activity. Most of the agents are natural or synthesized by bacteria or fungi (Table 1). In contrast, several important agents are completely synthetic (Sulphonamides, trimethoprim, chloramphenicol, ciprofloxacin, isoniazid and dapsone). While some are semi synthetic, natural antibiotics that have been chemically modified by the addition of extra groups to make them less susceptible to inactivation by pathogens. Ampicillin, carbenicillin, and methicillin are good examples.

**Vaccines and Antibiotics**

**Antibiotics** and **vaccines** are both used to fight germs but they work in different ways. While vaccines are used to prevent disease, antibiotics are used to treat diseases that have already occurred. In addition, antibiotics do not work on viruses or viral illnesses such as common cold or flu.

**Definitions**

**Antibiotics** are compounds that are effective in treating infections caused by organisms such as bacteria, fungi and protozoa. Antibiotics are mostly small molecules, less than 2000 Daltons.

**Vaccines** are compounds that are used to provide immunity to a particular disease. Vaccines are usually dead or inactivated organism or compounds purified from them.

**Differences in Sources**

**Antibiotics** can be derived from natural, semi-synthetic and synthetic sources and source of vaccines include live or inactivated microbes, toxins, antigens, etc.

**Vaccines** are usually derived from the very germs the vaccine is designed to protect against. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.

**History**

Even before the concept of germs and diseases was understood, people in Egypt, India and the natives in America used molds to treat certain infections. The first breakthrough in **antibiotics** came with the discovery of penicillin by Alexander Fleming in 1928. This was followed by the discovery of sulfa drugs, streptomycin, tetracycline, and many others antibiotics to combat different microbes and diseases.

The earliest reports of **vaccines** seem to have originated from India and China in the 17th century and recorded in Ayurvedic texts. The first description of a successful vaccination procedure came from Dr. Emmanuel Timoni in 1724, followed by Edward Jenner's independent description, half a century later, of a method for vaccinating humans against small pox. This technique was further developed by Louis Pasteur during the 19th century to produce vaccines against anthrax and rabies. Since then attempts have been made to develop more vaccines against many more diseases.

**Different Types of Antibiotics and Vaccines**

**Types of Antibiotics**

Classification according to effect on Bacteria

Antibiotics are mainly of two types, those that kill bacteria (bactericidal) and those that inhibit bacterial growth (Bacteriostatic). These compounds are classified according to their structure and mechanism of action, for instance antibiotics can target bacterial cell wall, cell membrane, or interferes with the bacterial enzymes or important processes such as protein synthesis.

**Classification based on source**

Besides this classification, antibiotics are also grouped into natural, semi-synthetic and synthetic types depending on whether it is derived from living organisms, like aminoglycosides, modified compounds like beta-lactams — e.g., penicillin — or purely synthetic, such as sulfonamides, quinolones and oxazolidinones.

**Classification based on bacteria spectrum**

Narrow spectrum antibiotics affect particular bacteria whereas large spectrum antibiotics affect a wide range of bacteria. In the recent years, antibiotics have been classified into three classes, cyclic lipopeptides, oxazolidinones and glycylcyclines. The former two are targeted at gram-positive infections whereas the last one is a broad spectrum antibiotic, treating many different types of bacteria.

**Types of Vaccines**

Vaccines are of different types-live and attenuated, inactivated subunit, toxoid, conjugate, DNA, recombinant vector vaccines and other experimental vaccines.

**Live, attenuated vaccines** are weakened microbes that help cause lifelong immunity by eliciting a strong immune response. A huge disadvantage of this type of vaccine is that because the virus is live, it can mutate and cause severe reactions in people with a weak immune system. Another limitation of this vaccine is that it has to be refrigerated to stay potent. Examples for this type include vaccines against chicken pox, measles and mumps.

**Inactivated vaccines** are dead microbes and safer than live vaccines, though these elicit a weaker immune response, and often have to be followed by booster shots. TheDTap and Tdap vaccines are inactivated vaccines.

**Subunit vaccines** include only subunits or antigens or epitopes (1 to 20) that can evoke an immune response. Example of this type includes vaccine against hepatitis C virus.

**Toxoid vaccines** are used in case of infections where organisms secrete harmful toxins in the body of the host. Vaccines with "detoxified" toxins are used in this type.

**Conjugate vaccines** are used for bacteria that possess a polysaccharide coating that is not immunogenic or recognized by the immune system. In these vaccines, an antigen is added to a polysaccharide coating to enable the body to produce an immune response against it.

**Recombinant vector vaccines** use the physiology of one organism and DNA of another to target complex infections.

**DNA vaccines** are developed by inserting the infective agent's DNA into human oranimal cell. The immune system is thus able to recognize and develop immunity against the organism's proteins. Though, this is still at the experimental stage, the effect of these types of vaccines promises to last longer and can be easily stored.

Other experimental vaccines include Dendritic cell vaccines, and T-cell receptor peptide vaccines.

**Administration of Vaccines vs. Antibiotics**

**Antibiotics** are usually given orally, intravenously or topically. The course may last from a minimum of 3-5 days or longer depending on the type and severity of the infection.

A large number of **vaccines** and their **booster shots** are usually scheduled before the age of two for children. In the United States, routine vaccinations for children include those against hepatitis A, B, polio, mumps, measles, rubella, diphtheria, pertussis, tetanus, chickenpox, rotavirus, influenza, meningococcal disease and pneumonia. This routine might differ in other countries and iscontinually being updated. Vaccinations for other infections such as shingles, HPV are also available.

**Side effects**

Though **antibiotics** are not considered unsafe, these compounds may cause certain adverse reactions. These include, fever, nausea, diarrhoea and allergic reactions. Antibiotics may cause severe reactions when taken in combination with another drug or alcohol. Antibiotics also tend to kill the "good" bacteria, whose presence in the body — especially the gut — is important for health.

**Vaccine safety**

There have been many disputes, over the effectiveness, and ethical and safety aspects of using **vaccines** in the past. For example, a study published in June 2014 in the Canadian Medical Association Journal found that the combination measles–mumps–rubella–varicella (MMRV) vaccine doubles the risk of febrile seizures in toddlers when compared with administration of separate MMR and varicella vaccines (MMR+V).

Under the National Childhood Vaccine Injury Act (NCVIA), federal law requires thatVaccine Information Statements (VIS) be distributed to patients or their parents whenever certain vaccines are administered. The CDC maintains that vaccines now produced meet very high safety standards so that the overall benefit and protection vaccines offer against diseases far outweighs any adverse reactions it might have in some individuals.

**Comparison chart**

Antibiotics versus Vaccines comparison chart

| | Antibiotics | Vaccines |
|---|---|---|
| **Definition** | Antibiotics are small molecules or compounds that are effective in treating infections caused by organisms such as bacteria, fungi and protozoa. | Vaccines are dead or inactivated organisms or compounds that are used to provide immunity to a particular infection or disease. |
| **Types** | Antibiotics are classified according to their structure and mechanism of action into 3 classes: cyclic lipopeptides, oxazolidinones & glycylcyclines. The first 2 are targeted at Gram positive infections and the last one is a broad spectrum antibiotic | Vaccines are of different types-live and attenuated (vaccines against chicken pox), inactivated (BCG vaccine), subunit (Hepatitis C), toxoid, conjugate, DNA , recombinant vector vaccines and other experimental vaccines. |

| | | |
|---|---|---|
| **Side effects** | Some antibiotics may have side effects like diarrhea, nausea and allergic reactions. | Some vaccines may cause allergic reactions. |
| **Source** | Antibiotics can be derived from natural, semi-synthetic and synthetic sources. | Sources of vaccines include live or inactivated microbes, toxins,antigens, etc. |

**Phage typing** is a method used for detecting single strains of bacteria. It is used to trace the source of outbreaks of infections. The viruses that infect bacteria are called bacteriophages ("phages" for short) and some of these can only infect a single strain of bacteria. These phages are used to identify different strains of bacteria within a single species.

A culture of the strain is grown in the agar and dried. A grid is drawn on the base of the petri dish to mark out different regions. Inoculation of each square of the grid is done by a different phage. The phage drops are allowed to dry and are incubated: The susceptible phage regions will show a circular clearing where the bacteria have been lysed, and this is used in differentiation.

**Bacteriocins** are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). They are similar to yeastand paramecium killing factors, and are structurally, functionally, and ecologically diverse. Applications of bacteriocins are being tested to assess their application as narrow-spectrum antibiotics.

Bacteriocins were first discovered by A. Gratia in 1925. He was involved in the process of searching for ways to kill bacteria, which also resulted in the development of antibiotics and the discovery of bacteriophage, all within a span of a few years. He called his first discovery a *colicine* because it killed *E. coli.*

**Possible Questions**

**Part B (Two marks)**

1. What is meant by Nosocomial?
2. Define pyrogen.
3. Write about type of UTI.
4. Define UTI.
5. Define RTI.
6. Define STD.
7. Define phage typing
8. Define bacteriocin typing.
9. Define vaccines.
10. Define antibiotics.

**Part C (Eight marks)**

1. What is generation of antibiotics?
2. Explain immunoprophylaxis.
3. Explain Nosocomial infection.
4. Comment on sexually transmitted disease.
5. Outline the symptoms of Respiratory tract infection.
6. Explain vaccination schedule.
7. What is antibiogram?
8. Write about the descending UTI?
9. Explain the ascending UTI.
10. Write about renal TB?

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS: I M.Sc MB** **COURSE NAME: MEDICAL BACTERIOLOGY**

**COURSE CODE: 19MBP202** **UNIT: V** **BATCH-2019-2021**

| S.No | Unit V | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | The common post operator consequence in hospital is _____ | Fever | gangrene | cold | sore | Fever |
| 2 | Semelweiss was able to control _____ in hospital by simpel hand washing | puerperal sepsis | meningitis | cholera | diarrhoea | puerperal sepsis |
| 3 | Lister overcome surgical infection by spraying _____. | acid | base | charcoal | phenol | phenol |
| 4 | The concept of asepsis _____ the incidence of hospital infection | reduce | elevate | . increase | improve | reduce |
| 5 | The incidence of hospital infection has been reported to be_____ | 10-20% | 12-20% | 2-12% | 10-15% | 2-12% |
| 6 | In hospital environment the normal flora is replaced by _____flora. | Drug resistant | common | Nosocomial | saprophyte | Drug resistant |
| 7 | Hospital acquired infection is also called as_____infection. | Nominal | Neutral | Normal | Nosocomial | Nosocomial |
| 8 | Hospital acquired infection are typically_____. | Exogenous | Endogenous | Epigenous | Eugenous | Exogenous |
| 9 | . _____ is diagnostic intervention in the hospital | Primary infection | re infection | post infection | Iatragenic infection | Iatragenic infection |
| 10 | The opportunity of a microorganism to infect patient is due to _____ impared. | Diet | immune | Invasive | Infection | immune |
| 11 | The hospital environment is heavily laden with wide variety of _____. | Commensals | Contaminants | Pathogen | Normal flora | Contaminants |
| 12 | The blister caused in bed return patient is _____. | Bed sore | Bedbug | Bedding | Bed wet | Bed sore |
| 13 | The slightest lapse in asepsis in hospital leads to _____. | Invention | Infection | Interaction | Innovatoin | Infection |
| 14 | _____ is the important cause of hospital infection | E.coli | Treponema | HIV | Streptococcus pyogenes. | Streptococcus pyogenes. |
| 15 | Staphylococcus of drug resistant belong to phage type _____. | 80/81 | 60/61 | 10/10/ | 44/10 | 80/81 |
| 16 | The drug resistance is _____ mediated. | Phage | Plasmid | Phasmid | cosmid | Phage |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | . _____ can grow in disinfectant. | E.coli | Klebsiella | Pseudomonas | Proteus | Pseudomonas |
| 18 | _____ spores can survive in cotton for long period | Tetanus | E.coli | Klebsiella | Bacillus | Tetanus |
| 19 | HIV is transmitted through _____ product. | Sputum | Urine | Blood | Stool | Blood |
| 20 | Viral infection are transmitted through_____ product | Pus | Blood | CSF | Swab | Blood |
| 21 | _____ the pathogen causes oral trush. | Candida | E.coli | Klebsiella | Cryptococcus | Candida |
| 22 | Stitch abscesses is _____ infection. | Brain | Wound | . CSF | Pus | Wound |
| 23 | Streptococcus wound infection manifest within a_____ period | Month | Year | Week | Day | Day |
| 24 | Clostridial wound infection manifest within a_____ period | Month | Year | Week | Day | Day |
| 25 | Pseudomonas cause infection in_____ | Burns | Wound | Brain | Blood | Burns |
| 26 | Neonatal tetanus have occurred due to the use of contaminated _____ | Blood | Body | Umblical cord | Brain | Umblical cord |
| 27 | Cathetrization cause _____ infection | UTI | RTI | CTI | systemic | UTI |
| 28 | About _____ of patient UTI common | 10 percent | 2percent | 5percent | 7percent | 2percent |
| 29 | E.coli and Proteus cause_____ infection. | Mixed | single | . combined | complicated | Mixed |
| 30 | _____ catheter are used in proper closed drainage | French | Glass | Indwelling | Rubber | Indwelling |
| 31 | Pulmonary ventilation may lead to nosocomial_____. | Pneumonia | . Bleeding | Abscesses | Fever | Pneumonia |
| 32 | Multiplication of bacteria in blood called_____ | Bacteria | Bacteremia | Viremia | Septicemia | Bacteremia |
| 33 | Multiplication of virus in blood called_____ | Bacteria | Bacteremia | Viremia | Septicemia | Viremia |
| 34 | viral infection are transmitted through_____ product | . Fungemia | Bacteremia | Viremia | Septicemia | Viremia |
| 35 | The liberation of toxin in blood is called_____ | Fungemia | Bacteremia | Viremia | Toximia | Toximia |
| 36 | Pus filled cavity called as_____. | Abscesses | Lesion | Necrosis | Fever | Abscesses |

| | | | | | | |
|---|---|---|---|---|---|---|
| 37 | Programmed cell death is called as_____ | Abscesses | . Lesion | Necrosis | Fever | Necrosis |
| 38 | Stool sample must be transported within _____ hour to lab. | 1 hour | 2 hours | 3 hours | 4 hours | 2 hours |
| 39 | Fever inducing agent is called as_____ | Pyrogen | Pyogen | Phelm | Parotid | Pyrogen |
| 40 | Phlebitis sets in with consequent _____. | Bacteria | Bacteremia | Viremia | Septicemia | Bacteremia |
| 41 | Staphylococcus epidermidis bacteremia is seen commonly in patient with artificial_____. | Skin | Catheter | Heart valve | Inhaler | Skin |
| 42 | Many hospital infection occur as_____ | Epidemic | Endemic | Pandemic | Randamic | Endemic |
| 43 | When out break occurs the source should be _____ | Cultured | Eliminated | Elevated | Intricate | Eliminated |
| 44 | An important contribution of microbiology to medical field is_____ | Immunization | Innovation | Infection | Intricate | Immunization |
| 45 | At sixth week _____ vaccination is given to child. | OPV | BCG | DPT | TT | BCG |
| 46 | At tenth week _____ vaccination is given to child | OPV | BCG | DPT | TT | DPT |
| 47 | At ninth month _____ vaccination is given to child | OPV | BCG | DPT | measles | measles |
| 48 | At 5-6 years _____ vaccination is given to child. | DT | BCG | DPT | TT | DT |
| 49 | At 10 years _____ vaccination is given to child. | DT | BCG | DPT | TT | TT |
| 50 | At 16 years _____ vaccination is given to child. | DT | TT | DPT | BCG | TT |
| 51 | Pregnant women should be given a booster dose of _____ | DT | BCG | DPT | TT | TT |
| 52 | OPV –O is given at _____ to child | At birth | Week | Month | Year | At birth |
| 53 | Booster dose help _____cells to remember immunological markers. | . B | T | Memmory | Plasma | Memmory |
| 54 | Fever of unknown origin is called_____ | Pyrexia | Pyrogen | Pyogen | Phylogenic | Pyrexia |
| 55 | Ulcer on finger is called as_____ | Boils | Tuleremia | Wound | Abscesses | Tuleremia |

| | | | | | | |
|---|---|---|---|---|---|---|
| 56 | Di dot is a test used for_____ | HIV | Rabies | Meningitis | Carditis | HIV |
| 57 | Inflammation of meninges is called_____ | Meningia | Meningitis | Peritonitis | Carditis | Meningitis |
| 58 | Arthritis is a _____ disorder | Immune | Auto immune | Mental | Blood | Auto immune |
| 59 | Allergic manifestation is called as_____ | Hypersensitivity | Hyposensitivity | . sensitivity | Histeria | Hypersensitivity |
| 60 | Topical ointments are used for _____ infection. | Bacteria | Bacteremia | Viremia | Septicemia | Bacteria |