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PATHOLOGY & MICROBIOLOGY

PATHOLOGY AND MICROBIOLOGY

Solved Question Papers

For Dental Students

Fifth Edition

Singi Yatiraj

JAYPEE

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BDS Second Year Examination December 2010

Revised Scheme 3

LONG ESSAYS

1. Classify clostridia. Write about the morphology, cultural characters and add a note on prophylaxis of tetanus.

- Clostridium consists of gram positive, anaerobic, spore forming bacilli.

Classification

- Clostridia are classified depending on the position of the spore (morphological) and their ability to ferment sugar and hydrolyze protein (biochemical).

Position of spores	Central/subterminal	Oval and terminal	Spherical and terminal
Predominately proteolytic	*Cl. bifermentans* *Cl. botulinum A*. B. F. *Cl. histolyticum* *Cl. sordelli* *Cl. sporogenes*	—	—
Predominately saccharolytic	*Cl. perfringens* *Cl. septicum* *Cl. chauvoei* *Cl. novyi*	*Cl. difficle*	—
Slightly proteolytic but not saccharolytic	—	—	*Cl. tetani*
Saccharolytic but not proteolytic	*Cl. fallax* *Cl. botulinum* C. D. E.	*Cl. tertium*	*Cl. tetanomorphum* *Cl. sphenoides*
Neither	--	*Cl. cochlearum*	--

Tetanus

- Tetanus is an infective musculoskeletal disorder characterized by tonic muscular spasms, usually commencing at site of infection and becoming generalized, involving the whole somatic muscular system
- Usually follows injury and rarely after surgical operations.

Incubation Period

- Two days to several weeks (6–12 days).

Causative Agent

- *Clostridium tetani*.

  Morphology
  - *Clostridium tetani* is a gram-positive slender bacillus about 4–8 μ × 0.5 μ
  - Have a straight axis, parallel sides and rounded ends

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- Occurs singly and occasionally in chains
- Spores are spherical, terminal and bulging giving bacillus characteristic "drum stick appearances"
- Noncapsulated and motile by peritrichate flagella.

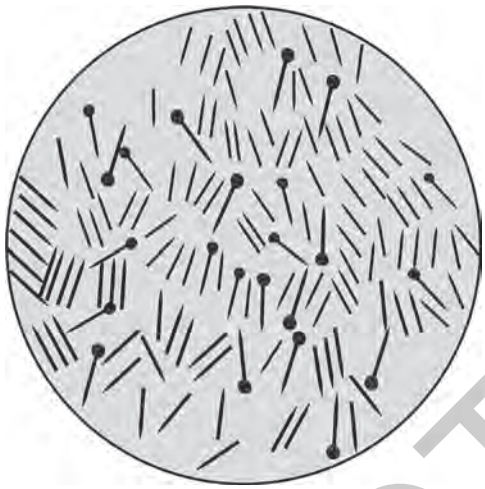


Fig. 1: Morphology of *Clostridium tetani*

Culture characteristics

- *Cl. tetani* is obligate anaerobe with optimum temp of 37°C and optimum pH of 7.4.

Culture media	*Growth characteristics*
• Blood agar	Swarming (thin spreading film) growth
• Horse blood agar	Colonies surrounded by zone of α-hemolysis, which subsequently develop into β-hemolysis due to production of tetanolysin, a oxygen labile hemolysin
• Cooked meat broth	Turbidity along with gas formation Meat is not digested but becomes black on prolonged incubation
• Deep agar shake cultures	Spherical fluffy balls, 1–3 mm in diameter, made up of filaments with a radial arrangement
• Gelatin stab cultures	Fir tree type of growth with liquefaction

Pathogenesis

- *Cl. tetani* has little invasive power
- Under favorable conditions such as reduced O-R potential, devitalized tissues, foreign bodies or concurrent infections, spores germinate and produce a toxin, tetanospasmin
- Toxin is absorbed by motor nerve endings and transported to central nervous system intra-axonally
- It specifically blocks presynaptic inhibition in spinal cord at inhibitory terminals that use glycine and GABA as neurotransmitter by inhibiting synthesis and liberation of acetylcholine
- Abolition of spinal inhibition causes uncontrolled spread of impulses initiated anywhere in CNS resulting in muscle rigidity and spasms due to simultaneous contraction of agonists and antagonists, in absence of reciprocal inhibition and convulsive pattern is determined by most powerful muscles at a given point
- Toxicity of tetanospasmin is influenced by route of administration, i.e. subcutaneous, intramuscularly and intravenous injections are equally effective whereas oral route is ineffective (toxin destroyed by digestive enzymes) but intraneural injections are most lethal
- Clinical picture is also determined by route of administration
- Intramuscular inoculation of toxin in limb produces tonic spasms of the muscles of inoculated limb (local tetanus) due to action of toxin on spinal cord segment containing motor neurons of specific nerve supplying inoculated area which is followed by spread of toxin up along spinal cord causing ascending tetanus involving opposite leg, trunk and arms
- Intravenous injection of toxin results in spasticity in head and neck muscles and then spreads downwards causing descending tetanus.

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Laboratory Diagnosis

Specimen

Excised bits of tissue from necrotic depths of wound.

a. Microscopy
   - Not reliable as typical drumstick's bacilli may be present in some wounds without tetanus developing and it is always not possible to distinguish morphologically between other similar bacilli such as *Cl. tetanomorphum* and *Cl. sphenoides*.

b. Culture
   - Specimen is inoculated on half of blood agar and incubated anaerobically for 1–2 days to demonstrate growth of attain bacilli in form of swarming growth on opposite half of agar plate
   - Moreover, specimen is inoculated into three tubes of cooked meat broth and to kill vegetative bacilli, one of test tubes is heated to 80°C for 15 minutes, second for 5 minutes and third left unheated and all three tubes are incubated at 37°C and subcultured on one half of blood agar plate daily up to 4 days and edges of swarming growth on agar are further subcultured to obtain pure culture
   - Addition polymyxin B to agar media makes it more selective.

c. Toxigenicity testing
   i. In vitro tests
      – *Cl. tetani* strains are stab inoculated in blood agar plates (4% agar) having tetanus anitioxins spread to one half of plate and incubated anaerobically for 2 days
      – Toxigenic *Cl. tetani* strains show hemolysis around the colonies only in half of agar plate without the antitoxin
      – Though this method detects tetanolysin but cannot indicate production of tetanospasmin, the main pathological toxin.
   ii. In vivo tests
      – Animal models are used to demonstrate production of tetanospasmin
      – 0.2 mL of 2–4 day old cooked meat culture is inoculated into root of tail of 2 mice
      – One mouse is a test mouse whereas an immunized mouse is used as control
      – Within 12–24 hours, test animal shows symptoms beginning with stiffness in tail
      – Rigidity proceeds to leg on inoculated side, opposite leg, trunk and forelimb in that order eventually leading to death of animal within 2 days.

Prophylaxis

a. Surgical prophylaxis
   - Aims at removal of foreign bodies, necrotic tissue and blood clots to prevent an anaerobic environment favorable for the tetanus bacillus
   - Range from simple cleaning of the wound or its radical excision.

b. Antibiotic prophylaxis
   - Aims at destroying or inhibiting the tetanus bacilli and any pyogenic bacteria in wound to prevent any toxin production
   - Consists of systemic administration of long-acting penicillin (drug of choice) or 500 mg erythromycin BD for 5 days and local application of bacitracin or neomycin.

c. Immunization
   i. Passive immunization (emergency procedure to be used only once)
      – Consists of injection of 1500 IU antitetanus serum SC or IM after testing hypersensitivity (inject 0.5 mL as trial dose SC and observe for ½ hours)
      – Alternatively 250 units of human antitetanus immunoglobulin may be used without risk of hypersensitivity.
   ii. Active immunization (most effective and only reliable method)
      – Consists of three spaced doses of formal toxoid (plain or adsorbed on aluminum hydroxide or phosphate) given intramuscularly as 0, 1, 6 months

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– This confers immunity for 10 years and requires a booster dose after that
– In person with previous immunization, only booster dose is sufficient.

iii. Combined immunization
– Consists of administration of human antitetanus immunoglobulin at one site and first dose of toxoid at the contralateral site followed by the 2nd and 3rd doses of toxoid at monthly intervals.

Summary of Tetanus Prophylaxis

Nature of wound	*Immune status of the person*		
	Immune (Full course of 3 toxoid)	Partially immune (2 injection of toxoid)	Nonimmune (0-1 injection of toxoid or unknown status)
Clean	1 toxoid	1 toxoid	3 toxoid
Contaminated	1 toxoid	1 toxoid HTIG Antibiotics	3 toxoid HTIG Antibiotics
Infected	1 toxoid Antibiotics	1 toxoid HTIG Antibiotics	3 toxoid HTIG Antibiotics

SHORT ESSAYS

2. Rhinosporidiosis.

- Rhinosporidiosis is a chronic granulomatous disease characterized by development of friable polyps, usually confined to nose, mouth or eye, but rarely seen on genitalia or other mucous membranes
- While disease is generally confined to mucous membranes, hematogenous dissemination may occur.

Causative Agent

- Rhinosporidium seeberi, a fungus*.

Morphologically and Life Cycle

i. Trophocytes
- Round or oval-shaped measuring 6–8 m with a cell membrane, clear cytoplasm, vesicular nucleus and a nucleolus
- Covered by chitin and lies freely in connective tissue

ii. Sporangia
- Trophocyte undergoes mitotic division, cytoplasm condenses, increases in size and wall thickens by developing into sporangium that contains endospores

iii. Endospores
- Each sporangium may contain 12,000–16,000 endospores having a thick outer chitinous wall and inner membranous wall with a germinal pore situated eccentrically
- Endospores may be released either through pore or rupture in sporangium

Pathogenesis

- Mode of infection is not known though infection is believed to originate from stagnant water or aquatic life.

Clinical Features

- Characterized by development of large friable polyps or wart like lesions
- Usually confined to nose, mouth or conjunctiva but rarely seen in ears, larynx, bronchus, penile urethra, vagina rectum and skin or other mucous membranes
- Generally confined to mucous membranes, hematogenous dissemination may occur to lungs, brain and bones.

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Laboratory Diagnosis

a. Microscopy
- Diagnosis is established by demonstration of sporangia on direct microscopy or histopathological examination
- Histologically, lesion is composed of large numbers of fungal spherules measuring 10–200 μm in diameter and containing thousands of endospores which are embedded in a stroma of connective tissue and capillaries.

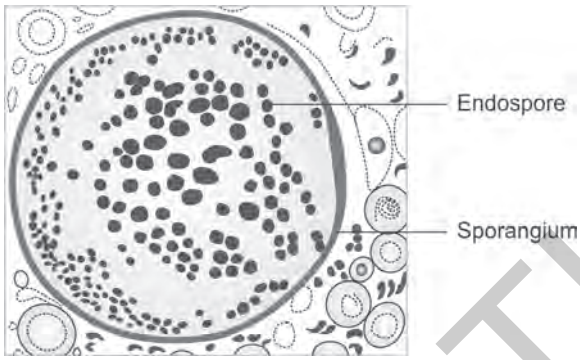


Fig. 2: Rhinosporidiosis

b. Culture
- *R. seeberi* cannot be cultivated.

3. Hot air oven.

Refer Question No. 2 June 2015 (RS3).

4. Erythrocytic shizogony.

- Erythrocytic schizogony is a stage in development of *Plasmodium* species wherein it passes through developmental stages of trophozoite, schizont and merozoite
- Also called merogony.

Erythrocytic Schizogony

- Merozoites released from pre-erythrocytic schizogony of *Plasmodium* species penetrate erythrocytes
- In erythrocytes, merozoites lose its interal organelles and appear as rounded body having a vacuole in center with cytoplasm pushed to periphery and nucleus at one pole and these young parasites are called ring forms (young trophozoites)
- As ring form develops, it enlarges in size becoming compact and black pigments collect into few 1–3 solid blocks and these are called late trophozoites
- When these trophozoites reaches certain stage of development, its nucleus starts dividing by mitosis followed by division cytoplasm to become mature schizonts or meronts
- A mature schizont of *P. falciparum* is 4.5 × 5 μm in diameter and fills 2/3rd of normal size erythrocytes
- It contains about 18–24 merozoites and hemozoin
- Red cells rupture releasing merozoites which attack new erythrocytes to continue their erythrocytic schizogony repeating cycle
- In *P. falciparum*, erythrocytic schizogony always takes place inside capillaries and vascular beds of internal organs, therefore in, *P. falciparum* infection schizonts and merozoites are not seen in peripheral blood but only ring forms are found.

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Significance

- This parasitic multiplication of parasite during erythrocytic schizogony is responsible for clinical attack of malaria.

SHORT ANSWERS

5. *Streptococcus mutans*.

- *Streptococcus mutans* is a viridans streptococci belonging to mutans group
- It is usually commensals of mouth and upper respiratory tract
- They are oridinarily nonpathogenic but on occasion can cause disease especially dental caries and subacute endocarditis.

Clinical Features

a. Dental caries
   - *Streptococcus mutans* adheres to dental surfaces via extracellular carbohydrates and erodes theeth by converting sucrose to acetic acid and lactate
   - It breaks down dietery sucrose producing acid and a tough adhesive dextran
   - The acid damages dentine whereas dextran binds together food debris, epithelial cells, mucus and bacterial forming dental plaques which leads to caries.

b. Subacute endocarditis
   - Subacute endocarditis following dental extraction is attributed to *Streptococcus mutans*
   - In patients with underlying valvular pathology, dental procedures, vigorous tooth brushing or chewing and use of a pick to clean teeth can cause transient bacteremia that is enough to initiate valvular infection
   - Therefore, most of the dental procedures in patients with valvular heart disease are undertaken under antibiotic cover.

6. Transduction.

Refer Question No. 4 June 2011 (RS).

7. Herpes simplex type I infections.

Refer Question No. 3 Dec. 2015 (RS3).

8. Name two functions of IgM.

Refer Question No. 7 Dec. 2012 (RS).

9. Dark-field microscope.

- Dark ground or dark-field microscopy is another method of improving the contrast which is applied for observing delicate organism such as spirochete which have a refractive index so close to that of the medium that they cannot be seen by routine oil immersion objectives.

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Three requirements for dark ground microscopy are:

i. A dark ground condenser with a central circular stop which allows a wide cone of light in excess to that required to fill the objective glass and check out that portion of the cone which would normally enter the front of the objective.
ii. Suitable illuminant of sufficient intensity.
iii. Numerical aperture less than 1.

Principle

- By means of the special condenser lens, the light is so arranged that the direct rays do not enter the tube of the microscope
- The oblique light rays are reflected or scattered on to the objective lens of the microscope, resulting in the object appearing self-illuminous against a dark background
- The preparation must be thin to have satisfactory results.

Applications

- For examination of spirochetes from lesion
- Demonstration of motility of other bacteria
- Examination of organisms without staining.

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