CHAPTER VIII

FAMILY SPIROCHAETACEAE AND LEPTOSPIRACEAE

Learning objective:

At the end of this chapter the students will be able to:

- List the medically-important species of Family Spirochaetaceae and Leptospiraceae
- Describe general characteristics of Family Spirochaetaceae and Leptospiraceae
- Recognize diseases caused by Family Spirochaetaceae and Leptospiraceae
- Describe the virulent factor of pathogenic species of Family Spirochaetaceae and Leptospiraceae
- Discuss pathogenicity, clinical manifestations, laboratory diagnosis, prevention & control of members of the Family Spirochaetaceae and Leptospiraceae

Family Spirochaetaceae

- Elongated, motile, flexible bacteria twisted spirally along the long axis are termed spirochetes
  - Speira meaning coil
  - Chaite meaning hair
- A characteristics feature is the presence of varying number of endo-flagella or internal flagella which constitute an axial filament
- Motile and vary widely in size
- A cell wall sharing many properties with Gram negative bacteria
- Sensitive to penicillin and metronidazol
- Consists two genera of major human pathogen; Treponema and Borrelia

Genus Treponema

General characteristics

- Helically coiled, corkscrew-shaped cells
- Motile, rapid rotation along its longitudinal axis
- Gram negative, but stain poorly
- Microaerophilic
- Multiply by binary transverse fission and with long in-vivo generation time (30hrs)
- Not yet been cultured

Genus Treponema include

- T. pallidium ssp pallidium
- T. pallidium ssp pertenue
- T. pallidium ssp endemicum
- T. carateum

Normal inhabitants

- T. pallidium ssp and T. carateum are obligate human pathogens
- Non pathogenic treponemes are part of the normal flora of intestinal tract, oral cavity or the genital tract

Virulence factors

- Outer Membrane Protein,
- enzymes (hyaluronidase)
- Perivascular inflammation
- Granulomatous changes: tertiary syphilis

Antigenic structure

1. Non – specific antigen

- A non-specific (reagin) appear in blood of syphilitic patients that reacts with a lipid hapten extracted from beef heart.
- The hapten is known as cardiolipin and is chemically a diphosphatidyl glycerol
- It is not certain whether cardiolipin is an antigen contained in T. pallidum itself or a product of damaged tissue caused by infection.

2. Specific Antigen

- **Group specific antigen**
  - It is a protein Ag present in T. pallidum as well as non – pathogenic treponems, such as reiter treponems
  - Antibody to this antigen appears in serum of syphilitic patients
  - The antigen used in tests to detect group specific antibody is derived from the reiter treponeme

- **Species – Specific treponemal antigen**
  - appears to be polysaccharide in nature
  - T. pallidium is used as antigen for detection of species – specific antibody
  - The antibody to this antigen is demonstrated by the specific T. pallidum tests, which are positive only with sera of patients infected with pathogenic treponems

Pathogenicity and Clinical features

- *T. pallidum* causes:
  - Sexually acquired syphilis which has
    - an early infectious stage occurring within the first 2 years of infection, and
    - a late non-infectious stage.
- *Early stage*: Includes primary syphilis, secondary syphilis and early latent syphilis.
- *Late stage*: Includes late latent syphilis, benign late syphilis, cardiovascular syphilis, and neurosyphilis.

Veneral treponematosis is caused by T. pallidium ssp pallidium

Primary Syphilis (21 days)

- hard chancre, a superficial non painful ulcer with a firm base, penis, labia, cervix, anorectal region, mouth: highly infectious
- Regional lymphadenopathy
- After 2-6 heals without treatment

Secondary syphilis

- 46-24 weeks: low grade fever generalized lymphadenopathy, malaise and a mucocutaneous rash
- Superficial sores on mouth, vagina, or anus
- Highly infectious lesions
- After 2-6 weeks healing occur
- 25% untreated experience recurrence

Latent syphilis

- Between secondary and tertiary
- When secondary relapse occur
- Cardiovascular syphilis (80%)
- Neurosyphilis (20%)
- In many cases, this is followed by natural cure but in others, after several years', manifestation of tertiary syphilis appear

Tertiary Syphilis

- It is a slowly progressive, destructive inflammatory disease that may affect any organ.
- It may lead to relatively benign ulcerating lesions of the skin, mucous membranes or bones, or gummata of the internal organs
- Consist of cardiovascular lesion including aneurysms and meningo vascular manifestation.
- The lesion of tertiary syphils contain very few organisms

Congenital syphilis

- This occurs when infection is transmitted from mother to offspring transparently.
- It may lead to abortions or still birth.

Non-veneral trepanomatoses

- *Treponema pallidum* subsp. *pertenue* causes yaws,
  - 1^0, 2^0, 3^0 yaws
- *Treponema pallidum* subsp. *endemicum* causes endemic syphilis (bejel),
- *Treponema carateum* causes pinta

Laboratory diagnosis

- **Specimen collection**
  - Tissue exudates, serous fluid, secretions for 1^0, 2^0, early congenital syphilis
  - Blood, CSF, plasma for 2^0, latent, 3^0 and late congenital syphilis
  - Specimen should be collected with care as the lesions are highly infectious

- **Microscopic examination**
  - Dark field, phase contrast
    - T. pallidum is identified by its slender spiral structure and slow movement
  - Direct Flourescent Antibody staining
    - They are treated with fluorescent tagged anti treponema pallidum antiserum
    - Observe under fluorescent microscope
    - The test can be made more reliable by using specific monoclonal antibody
  - Silver stain
  - Diagnosis by microscopy is done in primary and secondary stage and congenital syphilis with superficial lesion.
- **Culture:** can not be cultivated

- Serologic tests
  - Non specific treponemal tests
    - VDRL, RPR
    - The antigen used in these tests is an alcoholic extract of beaf heart tissue (cardiolipin) to which lecithin and cholesterol are added.
    - The major disadvantage in these tests is the antigen is non – specific and hence gives rise to biological false positive reaction
  - Specific treponemal tests
    - FTA-Abs (Fluorescent treponemal antibody absorption test)
    - TPHA (*T. pallidum* haemagglutination assay)
    - TPPA (*T. pallidum* particle agglutination assay)

Treatment

- Penicillin is the drug of choice

Prevention and control

- Treatment of cases
- Follow up of cases and contact
- Prophylactic use of sex barriers (such us condom)

Genus Borrelia

- Large, motile, refractile spirochetes with irregular, wide, open coil
- Transmitted to vertebrate hosts by haematophagous arthropode
- Gram Negative
- Stain by ordinary dye
  - Borreliae stain readily with bacteriologic dyes as well as with blood stains such as Giemsa's stain or Wright's stain
- The borreliae form irregular spirals.
- The distance between turns varies from 2μm to 4μm.

- The organisms are highly flexible and move both by rotation and by twisting.
- Medically important species include
  - Borrelia Vincenti
  - Borrelia recurrentis
  - Borrelia burgdorferi
  - Borrelia duttoni

Pathogenicity and clinical manifestation

- There are two forms of relapsing fever:
  - Louse-borne relapsing fever
  - Tick-borne relapsing fever
- Borreliae may also infect the central nervous system causing meningitis (lymphocytic) and occasionally the organisms can be seen in cerebrospinal fluid.

Louse-borne relapsing fever

- caused by *Borrelia recurrentis*, a human pathogen which is transmitted from person to person by the body and head louse, *Pediculus humanus*.
- Relapsing fever is characterized by recurring periods of high fever with severe headache, body pains, vomiting and often a cough and dyspnoea.
- There may be up to four relapses of decreasing severity at 5–9 day intervals.

Tick-borne relapsing fever

- caused by *Borrelia duttoni*
- is transmitted by soft ticks of the genus *Ornithodorus*.
- Humans are the main reservoir of *B. duttoni* but in some areas of the world, rodents are also important reservoirs of *Borrelia* species.
- Clinical manifestation similar to those described for louse-borne relapsing fever except tick-borne relapsing fever is usually a less severe illness.

Borrelia vincenti

- *B. vincenti* in association with *Fusobacterium* species and other Gram negative anaerobes causes:
  - acute ulcerative gingivitis (Vincent's angina)
  - tropical ulcer
- *B. burgdorferi* and other *Borrelia* species cause Lyme disease which is transmitted by hard ticks of the genus *Ixodes*.
- Field mice, voles, sheep, horses, and other mammals are among the reservoir hosts.
- Humans become infected by the bite of an infected hard tick.

Laboratory diagnosis

- ***Specimens*:**
  - Blood
  - cerebrospinal fluid

Microscopic examination

- *Borrelia* organisms are large spirochaetes, with uneven size coils.
- They can be seen in thick (and occasionally thin) blood smears stained with Giemsa or Field's stain
- Microscopically it is not possible to differentiate the different species of *Borrelia*.

Culture

- Culture is not used routinely as a method of diagnosing relapsing fever.
- However, it grow in Kelly’s medium

Serology

- Several serological tests have been developed for diagnosing relapsing fever, but antigens for the tests are not generally available and many show cross reactions with *Treponema*.

Treatment

- Erythromicin, tetracyclin, penicillin

Prevention and control

- avoidance of exposure to ticks and lice and on delousing (cleanliness, insecticides).

Genus Leptospira

- It is a single genus of family Leptospiraceae
- It contains 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.*

- Leptospirae are tightly coiled, thin, flexible spirochetes, with very fine spirals 0.1–0.2 m wide;
- one end is often bent, forming a hook.
- They are actively motile, which is best seen using a darkfield microscope.
- The spirochete is so delicate that in the darkfield view it may appear only as a chain of minute cocci.
- It does not stain readily but can be impregnated with silver.

Leptospira interrogans

Pathogenesis and clinical manifestation

- *L. interrogans* causes leptospirosis, a zoonotic disease with humans becoming infected following direct or indirect contact with urine from infected animals.
- The mild form of leptospirosis is often misdiagnosed as a viral illness or influenza.
- The more serious form of the disease is characterized by high fever, headache, hypotension, pains in the muscles and legs, abdominal pain, weakness, and often redness of the eyes and a rash.
- Some patients develop meningitis (lymphocytic), jaundice following liver cell damage, thrombocytopenia with haemorrhagic symptoms, and renal failure.

- ***Weil's disease, or syndrome*:**
  - This term is occasionally used to describe the severe form of leptospirosis in which there is liver damage with jaundice and renal failure.

Laboratory diagnosis

- ***Specimens*:**
  - Mainly blood for culture (First week)
  - Urine (second and third week)
  - Serum (second and third week)
  - CSF

Culture

- In Reference Laboratories, specimens can be cultured for *L. interrogans*.
- The organism can be grown aerobically at 28–30 ºC in a semi-solid serum culture medium or a Tween-albumin (TA) medium.
- Cultures are examined weekly for leptospires by dark-field microscopy.
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Serological tests

- This is the usual method of confirming leptospirosis
- Serological tests used to investigate leptospirosis can be divided into:
  - Tests which detect *Leptospira* specific antibodies
    - i.e. genus specific, such as *LeptoTek Dri-Dot* and *LeptoTek Lateral Flow*.
  - Microscopical agglutination test (MAT) which is used to identify the infecting serovar.

Treatment

- Penicillin is the drug of choice
- Doxycyclin, piperacillin, and cefotaxime are also effective

Prevention and control

- 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.