



31

SPIROCHAETES

31.1 INTRODUCTION

Elongated, motile, flexible bacteria twisted spirally along the long axis are termed 'spirochetes' (from *Speira*, meaning coil and *chaite*, meaning hair). It has two families: (1) *Spirochaetaceae* in which the spirochaetes are anaerobic, facultative anaerobic or microaerophilic and not hooked. This family includes the genera *Treponema* and *Borrelia* and (2) *Leptospiraceae* which is a family of hooked and obligate aerobic *spirochaetes*. The genus *Leptospira* is included in this family.

Structurally, the spirochetes have gram-negative-type cell wall composed of an outer membrane, a peptidoglycan layer and a cytoplasmic membrane. However, they are more complex than other bacteria. A characteristic feature is the presence of varying number of endoflagella. These endoflagella impart the shape and the following types of motions to the *Spirochaetes*:

- Flexion and extension.
- Cork-screw like rotatory movement along the long axis.
- Translatory motion i.e. from one site to another.



OBJECTIVES

After reading this lesson, you will be able to:

- discuss the characteristics of *Spirochaetes*.
- differentiate between the various groups of *Spirochaetes*.
- discuss the pathogenicity, laboratory diagnosis of Syphilis.
- discuss the features of *Borrelia* and *Leptospira*.



Notes

31.2 GENUS TREPONEMA

Treponemes are relatively short slender spirochetes with fine spirals and pointed or rounded ends. *Treponemes* cause the following diseases in humans:

- Venereal syphilis caused by *T pallidum*.
- Endemic syphilis caused by *T endemicum*.
- Yaws caused by *T pertenue*.
- Pinta caused by *T carateum*.

***Treponema pallidum*:** It is thin, delicate, spiral filament 6-14 μm by 0.2 μm , with pointed and tapering ends. It has 6-12 coils which are comparatively small, sharp and regular. *T pallidum* cannot be seen under the light microscope. Its morphology and motility can be seen under the dark ground or phase contrast microscope. It cannot be stained by ordinary bacterial stains, but can be stained by silver impregnation methods. Fontana's method is used for staining films and Levaditi's method for tissue sections. On prolonged Giemsa staining they stain pale pink.

31.2.1 Culture

Pathogenic *treponemes* cannot be grown in artificial culture media but are maintained by subculture in susceptible animals e.g. Nichol's strain has been maintained in rabbit testes for several decades. Cultivable *treponemes* such as *T phagedenis* (Reiter's strain - widely used as the antigen in group specific *treponemal* tests for the diagnosis of syphilis) and *T refringens* (Noguchi's strain) are non-pathogenic.

31.2.2 Diseases

Treponema pallidum leads to syphilis which is acquired by sexual contact. Other modes of transmission includes blood borne infections, congenital (from mother to child) and occupational. Incubation period ranges from 10 to 90 days. The clinical manifestations fall into three stages- primary, secondary and tertiary.

The primary stage is characterized by a hard, circumscribed, chancre, which is usually genital. Secondary syphilis sets in 1-3 months after the primary lesion heals. Roseolar or papular skin rashes, mucous patches in the oropharynx and condylomata at the muco-cutaneous junctions are the characteristic lesions. This may be followed by natural cure or in some cases by manifestations of tertiary syphilis. These include cardiovascular lesions including aneurysms, chronic granulomata (gummata) and meningovascular manifestations.

31.2.3 Laboratory Diagnosis

Laboratory diagnosis consists of demonstration of the spirochetes under the microscope and of antibodies in serum or CSF.

(i) Microscopy

- (a) *Dark ground microscopy*: Diagnosis by microscopy is applicable in primary and secondary stages and in cases of congenital syphilis with superficial lesions. Specimens should be collected with utmost care as the lesions are highly infectious. *T pallidum* is identified by its slender spiral structure and slow movement in dark ground illumination. Smears can be stained by silver impregnation method and visualized under light microscope.
- (b) *Direct fluorescent-antibody staining for T pallidum (DFA-Tp)*: The smear to be tested is stained with fluorescein-labelled pathogen specific monoclonal antibody. The treponemes appear distinct, sharply outlined and have apple-green fluorescence.

(ii) Serological tests

These are divided into non-treponemal tests (non-specific/standard test for syphilis) and treponemal tests.

- (a) *Non-treponemal tests/ standard tests for syphilis(STS)*: These tests are used as screening tests. Reagin antibodies are detected by cardiolipin antigen. Cardiolipin antigen is an alcoholic extract of beef heart tissue to which lecithin and cholesterol are added. Non-treponemal tests include
- Venereal Diseases Research Laboratory (VDRL)
 - Rapid Plasma Reagin test(RPR)
 - Tolidine Blue Unheated Serum Test(TRUST)
 - Wassermann complement fixation test
 - Kahn tube flocculation test

The Wassermann reaction and the kahn test are now replaced by the VDRL test. VDRL is the most widely used simple and rapid test. It is performed as a slide flocculation test, in which the inactivated patient serum is mixed with a freshly prepared suspension of cardiolipin-cholesterol-lecithin antigen on a glass slide. The result is read under low power objective of microscope. Formation of visible clumps or floccules is taken as positive reaction. In case of negative result, the antigen particles are seen as evenly dispersed small fusiform needles. The test is performed both as qualitative and quantitative assay. VDRL test can be used for testing CSF also but not plasma. The major disadvantages of the VDRL test are the need to prepare fresh antigen each day and to use microscope to read the results.



Notes



Notes

Most laboratories now use RPR test which employs a stabilized VDRL carbon antigen, which can be stored for upto 6 months at 4-10°C. This test does not require heat inactivation of patient's serum and can be read by naked eye.

TRUST is similar to RPR with an added advantage of storage at room temperature of 26-31°C.

The major disadvantage of standard test for syphilis is the biological false positive reactions (BFP), because of sharing of cardiolipin antigen of *T pallidum* and mammalian tissues.

BFP are defined as positive reactions obtained in cardiolipin tests, with negative results in specific treponemal tests, in the absence of past or present treponemal infections. They represent non-treponemal cardiolipin antibody responses. They can be classified as acute or chronic. Acute BFP reactions last for a few weeks or months and are associated with acute infections, injuries or inflammatory conditions. Chronic BFP reactions persist for more than 6 months and are seen in:

- SLE and other collagen diseases
- Leprosy
- Malaria
- Relapsing fever
- Infectious mononucleosis
- Hepatitis
- Tropical eosinophilia

(b) Treponemal tests for syphilis:

- Tests using cultivable treponemes: RPCF (Reiter protein complement fixation test)
- Tests using pathogenic treponemes:
 - (i) *Treponema pallidum* immobilization test
 - (ii) *Treponema pallidum* agglutination test
 - (iii) *Treponema pallidum* immune adherence (TPIA) test
 - (iv) Fluorescent treponemal antibody test (FTA)
 - (v) *Treponema pallidum* haemagglutination test (TPHA)
 - (vi) Enzyme immunoassay (EIA)

31.2.4 Treatment

Penicillin-G intravenous is the drug of choice.

Non-venereal Treponematoses: Occur in communities with poor standards of hygiene. Usually transmitted by direct body to body contact.

Spirochaetes

Non-venereal treponematosi	Endemic Syphilis	Yaws	Pinta
<i>Treponema species</i>	<i>T pallidum</i> subsp <i>endemicum</i>	<i>T pallidum</i> subsp <i>pertenue</i>	<i>T pallidum</i> subsp <i>carateum</i>
Endemic regions	Middle east, Zimbabwe, eastern Europe, India	Tropical areas of Asia, Africa and America, India	Central and South America
Synonyms	Bejel, njovera, Sibbens	Frambesia, pian, parangi	Carate
Transmission	Direct contact	Direct contact, flies may act as mechanical vectors	Direct contact
Primary lesion	Usually not seen. May be present on nipples of mother infected by their children	Extragenital papule which enlarges and breaks down to form an ulcerating granuloma	Extragenital papule which does not ulcerate but develops into a lichenoid or psoriaform patch
Secondary and tertiary lesions	Similar to syphilis	Similar to syphilis	Hyper and hypo pigmented patches
Laboratory diagnosis and treatment	Similar to syphilis.	Similar to syphilis.	Similar to syphilis.

MODULE

Microbiology



Notes

31.3 BORRELIA

Borrelia are large, motile, refractile spirochetes with irregular, wide, open coils. They are usually 5-30 μm long and 0.3-0.7 μm wide. They are motile and are readily stained by ordinary stains and are gram negative. A number of species, although fastidious, can be cultured. These are transmitted to vertebrate hosts by haematophagous arthropods. Important pathogenic species of *Borrelia* include: *B recurrentis* (relapsing fever), *B burdorferi* (lyme disease) and *B vincenti* (Vincent's angina).

- (a) *B recurrentis*: Relapsing fever is characterized by the occurrence of one or more relapses after the subsidence of primary febrile paroxysm. It is of two types; epidemic or louse borne, caused by *B recurrentis* and endemic or tick-borne relapsing fever caused by a number of species like *B duttoni*, *B hermsii*, *B parkeri*.

Borrelia causing relapsing fever can be seen by light microscopy in preparations stained by aniline dyes such as Wright or Geimsa. These can be demonstrated in the peripheral blood by direct stain. In fresh blood, they are actively motile with forward, backward and corkscrew like motions.



Diagnosis is by direct examination of wet films by dark ground or phase contrast microscopy or staining blood films with giemsa or gram stain using dilute carbol fuchsin as counter stain. Culture is too difficult and serology is unreliable. Tetracyclines, chloramphenicol, penicillin and erythromycin are effective.

- (b) *Borrelia burgdorferi*: These are flexible, helical and gram-negative. It is microaerophilic and can be cultured on BSK (barbour stoenner kelly) medium. It leads to Lyme disease. Transmission is by ixodid ticks. After an incubation period of 3-30 days, the patient develops a localized infection in the form of an expanding annular skin lesion (erythema migrans). This is followed by second stage of disseminated infection, wherein the patient develops fever, headache, arthralgia and lymphadenopathy. Third stage is of persistent infection, with chronic arthritis, polyneuropathy, encephalopathy and acrodermatitis. Diagnosis is by direct examination of skin lesions, blood or CSF by giemsa or gram stain using carbol fuchsin as counterstain. Darkground or fluorescent microscopy can also be done. Serological tests like ELISA and immunofluorescence have been described, confirmation is by immunoblotting. Doxycycline, amoxicillin and cefuroxime are useful for treatment.
- (c) *Borrelia vincenti*: *Borrelia vincenti* is a motile spirochete, with 3-8 coils of variable size. It is easily stained with dilute carbol fuchsin and is gram negative. It can also be stained by methyl violet, giemsa and leishman stains. It is an obligate anaerobe and can be cultured in sealed tubes containing digest broth enriched with ascetic fluid. In a symbiotic association with *Leptotricha buccalis*, *B. vincenti* leads to ulcerative gingivostomatitis or oropharyngitis (vincent's angina). For diagnosis, smears are made from the ulcerative lesions and are stained with dilute carbol fuchsin.

31.4 LEPTOSPIRA

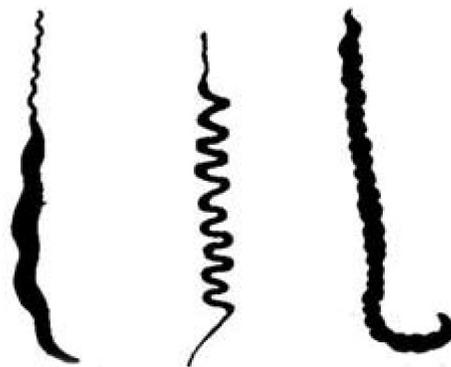
Leptospire are actively motile, delicate spirochetes, possessing a large number of fine and tightly coiled spirals and hooked ends like umbrella handles. They cannot be seen under the light microscope. They stain poorly with aniline dyes. They may be stained with Giemsa stain. Better results are obtained by silver impregnation methods. The genus *Leptospira* is classified into two species: *L. interrogans* containing pathogenic leptospire and *L. biflexa* containing saprophytic leptospire found predominantly in surface waters. Within each species are serogroups, which are further classified into serotypes (serovars).

Leptospire are obligate aerobes. Several liquid and semi-solid media, such as Korthof's, Stuart's and Fletcher's media have been described. Semisynthetic media such as EMJH (Ellinghausen, McCullough, Johnson and Harris) are now commonly used.

Spirochaetes

L. interrogans causes a zoonotic disease, transmitted to humans by direct or indirect contact with water contaminated by urine of carrier animals. Leptospirosis can be in the form of a mild febrile illness or sometimes the patient may land up into severe illness with jaundice and albuminuria, known as weil's disease.

Diagnosis of Leptospirosis can be done by direct microscopic examination of blood and urine. Serological tests used for diagnosis can be genus specific (complement fixation test, haemagglutination test, ELISA, etc) or serotype specific (microscopic and macroscopic agglutination test). Leptopires are sensitive to penicillin, tetracycline and erythromycin.



(a)

(b)

(c)

(a) = Borrelia

(b) = Spirochete

(c) = Leptospira



INTEXT QUESTIONS 31.1

- is the most widely used simple and rapid test for syphilis.
- Syphilis is acquired by contact.
- Vincent's angina is caused by
- B. burgdorferi* is cultured in
- Leptospira* are obligate
- Leptospira* causes severe form of illness known as
- Match the following:
 - Veneral syphilis (a) *T. endemicum*
 - Endemic syphilis (b) *T. carateum*
 - Pinta (c) *T. pallidum*
 - Yaws (d) *T. pertenu*

MODULE

Microbiology



Notes



WHAT YOU HAVE LEARNT



Notes

- Spirochetes are elongated, motile, flexible bacteria twisted spirally along the long axis.
- They are divided into two families: *Spirochaetaceae* in which the spirochaetes are anaerobic, facultative anaerobic or microaerophilic and not hooked.
- This family includes the genera *Treponema* and *Borrelia* and *Leptospiraceae* which are obligate aerobes and have hooked ends. This includes the genera *Leptospira*.
- Spirochetes have characteristic motility with flexion, extension and cork screw like movement.
- *Treponema pallidum* causes a venereal disease syphilis.
- Direct examination by dark ground microscopy can be done for the diagnosis of syphilis.
- For serology various non-treponemal and treponemal tests are available.
- The non-treponemal tests are also known as non-specific or standard test for syphilis. These are used as screening tests and include VDRL, RPR, TRUST.
- The treponemal tests are more specific. These include TPI (*T pallidum* immobilization test), TPA (*T pallidum* agglutination test), TPIA (*T pallidum* immune adherence test), FTA (fluorescent treponemal antibody test), FTA-ABS (fluorescent treponemal antibody-absorption test) and TPHA (*T pallidum* haemagglutination assay).
- The non-venereal species of *Treponema* include *T pallidum* subsp *endemicum*, *T pallidum* subsp *pertenue* and *T pallidum* subsp *carateum* which causes endemic syphilis, yaws and pinta respectively. They occur in communities with poor standards of hygiene and are usually transmitted by direct body to body contact.
- *Borrelia* are large, motile, refractile spirochetes with irregular, wide, open coils. They are motile and are readily stained by ordinary stains and are gram negative. A number of species, although fastidious, can be cultured. These are transmitted to vertebrate hosts by haematophagous arthropods. Important pathogenic species of *Borrelia* include: *B recurrentis* (relapsing fever), *B burdorferi* (lyme disease) and *B vincenti* (Vincent's angina).
- Leptospire are actively motile, delicate spirochetes, possessing a large number of fine and tightly coiled spirals and hooked ends like umbrella handles. They cannot be seen under the light microscope. Leptospire are

Spirochaetes

obligate aerobes and can be cultured in artificial media. *L interrogans* causes a zoonotic disease, which is transmitted by rodents. It can be manifested as a febrile illness or may be in the form of weil's disease with jaundice and albuminuria.



TERMINAL QUESTIONS

1. What are the characteristics of spirochetes?
2. Describe the Standard tests for syphilis.
3. Discuss the laboratory diagnosis of syphilis.
4. Discuss the pathogenicity of *Borrelia*.
5. Write a short note on relapsing fever.
6. Write briefly about vincent's angina.
7. Write a short note on Leptospirosis.
8. Differentiate between *Treponema*, *Borrelia* and *Leptospira*.



ANSWERS TO INTEXT QUESTIONS

31.1

1. VDRL
2. Sexual
3. *Borrelia Vincenti*
4. Barbour stoenner Kelly
5. Aerobes
6. Weil's disease
7.
 1. (c)
 2. (a)
 3. (d)
 4. (b)

MODULE

Microbiology



Notes