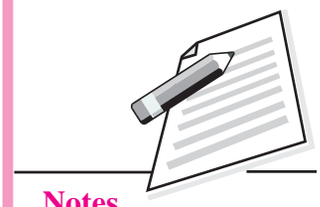




# 18

# NISSERIAE

## 18.1 INTRODUCTION

The Neisseria are Gram-negative cocci that usually occur in pairs. They are aerobic, nonsporulating, nonmotile, oxidase-positive cocci typically arranged in pairs. *N.meningitidis and N. gonorrheae* are medically important pathogens, and are found associated with or inside polymorphonuclear cells. Some Neisseriae sp are normal inhabitants of the human respiratory tract.

## OBJECTIVES

After reading this lesson, you will be able to:

- explain the characteristics of *Neisseria* species
- discuss the diagnosis of disease caused by *Neisseria* species

## 18.2 NEISSERIA MENINGITIDIS

### Morphology

Meningococci are Gram-negative, oval or spherical cocci, 0.6 – 0.8 µm in size, typically arranged in pairs, with the adjacent sides flattened.

### Cultural characteristics

Meningococci have exacting growth requirements and do not grow on ordinary media. Growth occurs on media enriched with blood, serum or ascetic fluid, which promote growth by neutralizing certain inhibiting substances in culture media rather than by providing additional nutritional needs.

Notes



They are strict aerobes, no growth occurs anaerobically. The optimum temperature for growth is 35-36°C. no growth takes place below 30°C. Optimum pH is 7.4-7.6. Growth is facilitated by 5-10 percent $CO_2$ and high humidity.

On solid media after incubation for 24 hrs, the colonies are small translucent, round, convex, bluish grey, with a smooth glistering surface and with entire edges. Blood agar, chocolate agar and Mueller-Hinton starch casein hydrolysate agar are the media commonly used for culturing meningococci.

## Biochemical reactions

They are catalase and oxidase positive, the prompt oxidase reaction helps in the identification of neisseria in mixed cultures. When a freshly prepared 1% solution of oxidase reagent is poured on the culture media, the neisseria colonies turn deep purple. Indole and hydrogen sulphide are not produced and nitrates are not reduced. Glucose and maltose are utilized, but not sucrose or lactose, producing acid but no gas.

## Antigenic properties and classification

Meningococci are capsulated, unlike other neisseriae. Based on their capsular polysaccharide antigens, meningococci are classified into at least 13 serogroups, of which Groups A,B and C are most important. Group A is usually associated with epidemics and Group C mostly with localized outbreaks, while Group B caused both epidemics and outbreaks.

## Resistance

Meningococci are very delicate organisms being highly susceptible to heat, dessication, alterations in pH and to disinfectants. They are sensitive to penicillin and other antibiotics, but resistance strains have emerged and become common in many areas.

## Pathogenicity

Cerebrospinal meningitis and meningococcal septicemia are the two main types of meningococcal disease. Meningococci are strict human parasites inhabiting the nasopharynx. Infection is usually asymptomatic. In some, local inflammation ensues, with rhinitis and pharyngitis. Dissemination occurs only in a small proportion. Most common complication include Waterhouse-Friderichsen syndrome, a massive, usually bilateral hemorrhage into the adrenal glands caused by fulminant meningococcemia, adrenal insufficiency and disseminated intravascular coagulation.



## Laboratory diagnosis

In meningococcal meningitis, the cocci are present in large numbers in the spinal fluid and, in the early stage in the blood as well. Demonstration of meningococci in the nasopharynx helps in the detection of carriers.

### (a) Examination of CSF

The fluid will be under pressure and turbid, with a large number of pus cells. For bacteriological examination, if a sufficient quantity is available, the CSF is divided into three portions. One portion is centrifuged and Gram- stained smears are prepared from the deposit. Meningococci will be seen mainly inside polymorphs but often extracellularly also. The second portion of the CSF is inoculated iin blood agar or chocolate agar plates and incubated at 35-36$^{o}$C under 5-10% $CO_2$. Colonies appear after 18-24 hrs which may be identified by morphological and biochemical reactions. The third portion of the CSF is incubated overnight either as it is or after adding an equal volume of glucose broth and then subcultured on chocolate agar.

### (b) Blood culture

Meningococcemia and in early cases of meningitis, blood culture is often positive. Cultures should be incubated for 4-7 days, with daily subcultures.

### (c) Nasopharyngeal swab

This is useful for the detection of carriers. The swab should be held in a suitable transport medium like stuart's medium

### (d) Petechial lesions

Meningococci may sometimes be demonstrated in petechial lesions by microscopy and culture.

### (e) Molecular diagnosis

Group-specific diagnosis of infection can be made by detection of meningococcal DNA sequence in CSF or blood by PCR amplifications.

## Treatment

Prompt treatment is essential to ensure recovery without sequelae. Intravenous penicillin G is the treatment of choice. Chloramphenicol is equally effective.

## INTEXT QUESTIONS 18.1

1. Neisseria are gram ..... cocci which occur in .....
2. ..... & ..... are pathogenic strains of Neisseria

Notes



3. N.gonorrhoeae commonly causes ..... in neonates
4. Common CNS infection Neisseria causes is .....

## 18.3 NEISSERIA GONORRHOEAE (GONOCOCCUS)

### Morphology

The organism appears as a diplococcus with the adjacent sides concave, being typically kidney shaped. It is predominantly within the polymorphs. Gonococci possess pilli on their surface. Pili facilitate adhesion of the cocci to the mucosal surfaces and promote virulence by inhibiting phagocytosis.

### Cultural characteristics

Gonococci are more difficult to grow than meningococci. They are aerobic but may grow anaerobically also. Growth occurs best at pH 7.2-7.6 and at a temperature of 35-36$^0$c with 5-10% CO2. They grow well on chocolate agar and Mueller-Hinton agar. A popular selective medium is the Thayer-Martin medium which inhibits most contaminants including nonpathogenic neisseria. Colonies are small, round, translucent, convex and slightly umbonate, with a finely granular surface and lobate margins.

### Biochemical reactions

Gonococci resemble meningococci except in the effect of maltose. Gonococci acidify only glucose and not maltose.

### Antigenic properties

Gonococci are antigenically heterogeneous. They are capable of changing their surface structures in vitro. Pili, which are hair like structures act as virulence factors by attaching to host cells and inhibiting phagocytosis. The trilaminar outer membrane of gonococci contains protein I and II which acts as ligands attaching the coccus to the host cells. The outer membrane also contain lipopolysaccharide which may be responsible for the toxicity in gonococcal infections.

### Resistance

The gonococcus is a very delicate organism, readily killed by heat, drying and antiseptics. In cultures, the coccus dies in 3-4 days but survives in slant cultures at 35$^o$C.



## Pathogenicity

Gonorrhea is a venereal disease which has been known since ancient times. The name gonorrhea, meaning flow of seed. The disease is acquired by sexual contact. Infection of the lower genital tract can result in a purulent or pus like discharge from the genitals which may be foul smelling. *N.gonorrhoeae* can also cause conjunctivitis, pharyngitis, proctitis or urethritis, prostatitis and orchitis. Conjunctivitis is common in neonates and silver nitrate or antibiotics are often applied to their eyes as a preventive measure against gonorrhea. Infection of the genitals in females with *N.gonorrhoeae* can result in pelvic inflammatory disease if left untreated, which can result in infertility.

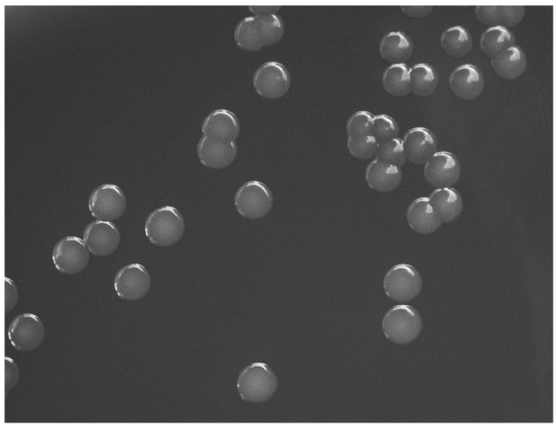


**Fig. 18.1**

## Laboratory diagnosis

### Specimens

Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear.

### Smears

Gram-stained smears of urethral or endocervical exudates reveal many diplococci within pus. These give a presumptive diagnosis.

### Cultures

Immediately after collection, pus or mucus is streaked on enriched selective medium like modified Thayer-Martin medium and incubated in an atmosphere containing 5% $CO_2$ (candle jar) at 36$^{o}$c. to avoid overgrowth by contaminants, the selective medium contains antimicrobial drugs like vancomycin, colistin, amphotericin. Forty-eight hours after culture, the organisms can be quickly

identified by their apperancce on Gram-stained smear, by oxidase positivity, and by coagglutination, immunofluorescence staining, or other laboratory test. The species of bacteria may be determined by rapid carbohydrate utilization tests.

### Nucleic Acid Amplification Tests

Several food and drug administration cleared nucleic acid amplification assays are available for detection of *N gonorrhoeae* in genitourinary specimens.

### Treatment

Penicillin G for inhibition (MIC - 2µg/ml). Pencillinase producing *N gonorrhoeae* (PPNG) also have increased in prevalence. Uncomplicated genital or rectal infections are treated with ceftriaxone 250mg given intramuscularly as a single dose. Additional therapy with azithromycin 1 gm / doxycycline, orally twice a day for 7 days, is recommended for the possible concomitant chlamydial infection.

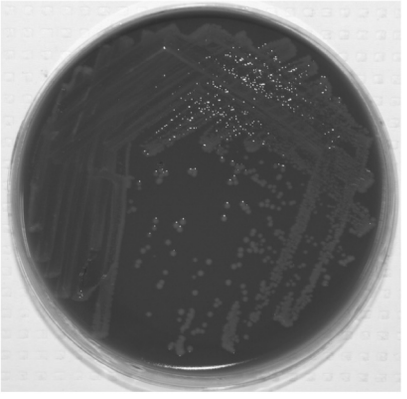
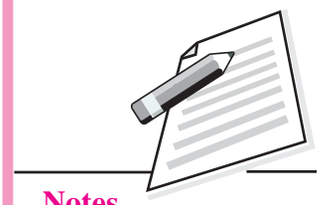


**Fig. 18.2**

## WHAT YOU HAVE LEARNT

- Neisseriae are gram-negative cocci occurring in pairs they are non-motile. Facultative aerobes that are catalase and oxidase positive
- *Neisseriae gonorrhoeae* (gonococci) and *Neisseria meningitidis* (Meningococci) are pathogenic for humans. Some neisseriae are normal inhabitants of respiratory tract, rarely causing disease
- *Neisseriae meningtidis* commonly cause of meningitis and septicemia
- *Neisseriae gonorrhoeae* causes gonorrhea, conjunctivitis, pharyngitis, proctitis, urethritis and prostatitis.





## TERMINAL QUESTIONS

1. Explain laboratory diagnosis of *Neisseria*
2. Describe the gold standard diagnosis of *N. Meningitidis*

## ANSWERS TO INTEXT QUESTIONS

1. Negative & Pairs
2. *Neisseria gonorrhoeae* and *Neisseria meningitidis*
3. Conjunctivitis
4. Meningitis