

Pyogenic cocci

Neisseria

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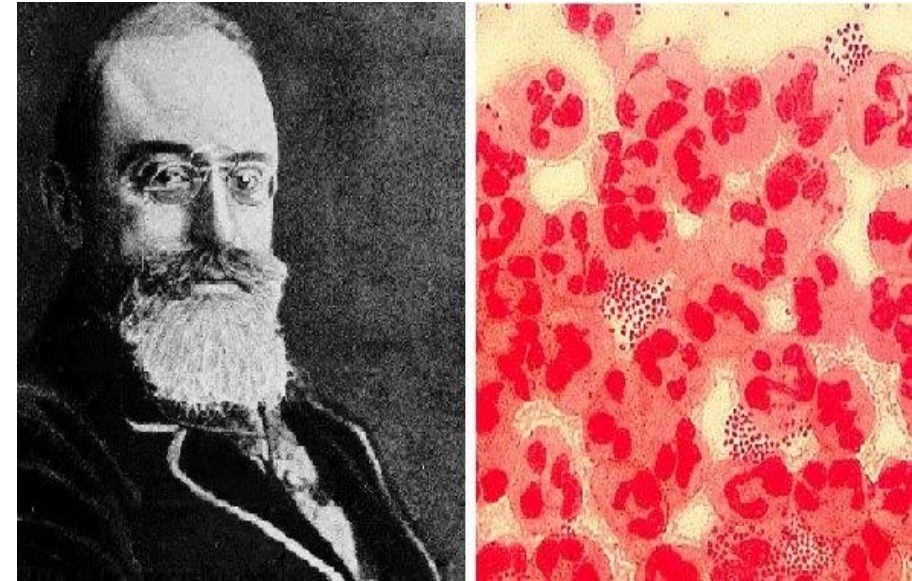
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General characteristics

These organisms are named in honor of **Dr. Albert Neisser** who in 1879 discovered the organism causing gonorrhoea.

- Gram negative, bean-shaped, diplococci
- Capsulated and possess pili
- Aerobic, oxidative metabolism
- Pathogenic species need enriched media and CO₂
- Catalase positive, oxidase positive
- Strict parasites



The genus contained about **30 species** that occur as commensals in the **mouth** and **upper respiratory tract**. The most important species are:

1/ Neisseria gonorrhoeae (gonococcus)

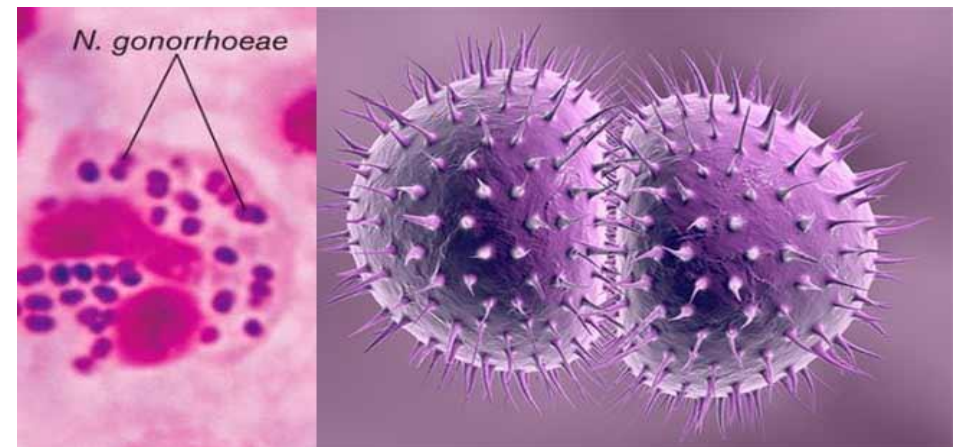
2/ Neisseria meningitidis (meningococcus)

cause a number of contagious infections of human columnar and transitional epithelium and hence produce urethritis cervicitis, salpingitis ,vulvovaginitis in children and ophthalmia neonatorum in new born.



Morphology

- In preparations made from gonorrhoeal pus, the gonococcus occurs in **pairs** or **intracellular diplococcus** resembling a pairs of kidney or beans. The long axis of the cocci in pairs are parallel and not in line.
- It is capsulated, non spore forming, non flagella, and non motile
- 0.8 -0.6 μm in diameter.



Cultural characters

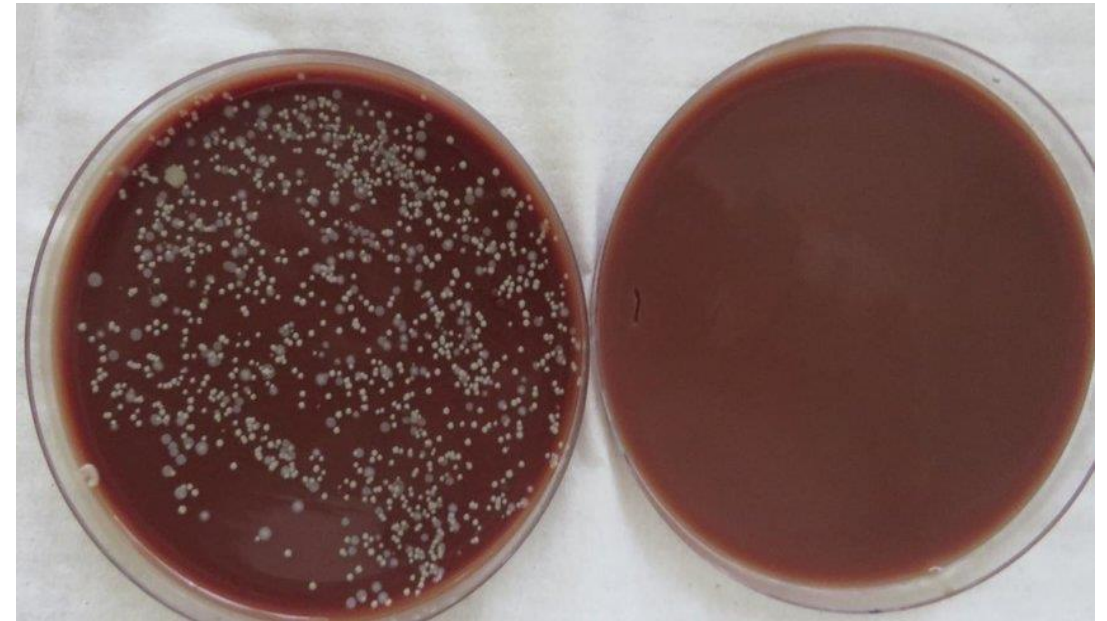
- Primary cultivation of the gonococcus on laboratory media is difficult, not only because the organism is fastidious in its growth requirements but also because it is **highly susceptible** to the toxic effects of a variety of substance commonly present in **ordinary culture** media.
- Most strains require an atmosphere containing 5-10% CO₂ to initiate development.



- A sufficient supply of **moisture** is essential for better growth.
- Best growth is obtained on **blood agar** and **chocolate agar**
- No haemolysis on blood agar
- On these media the **colonies are 1-2mm**
translucent, shining, viscid, convex, grey and
slightly granular surface
- Colonies of gonococcus are **smaller** than these of meningococcus and are more
viscous and **emulsify less readily**



- An effective selective medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis* has been developed by **Thayer and Martin (1966)** which contains antibiotic **Vancomycin**, **Colistin** and **Nystatin** in the chocolate agar.
- Vancomycin inhibits gram positive bacteria
- Colistin inhibits gram negative bacteria
- Nystatin for the inhibition of yeast



- The medium permits the growth of **pathogenic Neisseria** while inhibiting **saprophytic species** such as *N. sicca*, *N. catarrhalis*, *N. flava* and also prevents the growth of bacterial contaminants encountered in cervical, vaginal, urethral and even rectal specimens.
- Colonies may be slow in growth may not appear for 2-3 days.
- Inoculation of material to be cultured should if possible be made directly from the patient on to suitable medium pre-warmed to 37°C and the culture should be incubated at once.



Biochemical reactions

- The gonococcus ferments **glucose** and **not maltose** without gas, catalase, positive oxidase positive

Virulence factors

- **lipopolysaccharide endotoxin**, failure to detect significant levels of antibodies because the infection stimulates low level of antibody due to toxin local character.
- **Outer membrane proteins (OMP)** (attachment)
- **Pili** (adhesion, agglutination, inhibit phagocytosis)
- **IgA protease (IgA, IgG)**



Classification

- The gonococcus is divided into four types colonial appearance
- **T1 and T2**
 - Small brown colonies
 - Piliated
 - Autoagglutination
 - Virulent
- **T3 and T4**
 - Large granular, Non pigmented
 - Not piliated
 - No autoagglutination
 - Avirulent



Pathogenicity

Cause disease only in human, no part of normal flora, **transmitted by sexually contact** in both males and females

- **In male:** urethritis, epididymitis, seminal vesiculitis, prostatitis
- **In female:** urethritis, cervicitis, endometritis, salpingitis, peritonitis
- **In children:** vulvovaginitis, conjunctivitis
- **In new born:** ophthalmia neonatorum



Laboratory examinations

The discharge from the gonococcal lesions such as **blood, urethral discharge** in male, **cervical discharge** in female is subjected to the following examinations:

- 1- Microscopic examinations
- 2- Cultural examination
- 3- Oxidase reaction
- 4- Biochemical reaction fermentation test mannitol, not maltose
- 5- Immunofluorescent method
- 6- Serodiagnosis



Treatment

- No vaccine available present
- Penicillin or tetracycline
- Also can use cephalosporins and fluroquinolones

Prevention

Involved in safety measures (awareness)



Neisseria meningitidis (meningococcus)

Morphology

- **Gram negative** oval or spherical diplococcus
- with adjacent surfaces flattened ,the long axis of the cocci in pairs are parallel and not in line as in the case of pneumococci
- **Autolysis** is a prominent feature of meningococci
- Non motile bacteria and non spore former
- Groups A, C and D may form capsules
- Maltose fermenter, No beta lactamase



- **Strict aerobes** and primary cultures are obtained better in the presence of **5-10 % CO₂**.
- **Highly fastidious** optimum growth temperature is **35-37C**.
- **Chocolate agar ,blood agar ,trypticase soya agar and starch casein hydrolysate agar** are solid common media use for the propagation of these organisms.
- On chocolate and blood agar the colonies are **moist, elevated smooth, semitransparent** often 48hrs incubation, the center of the colonies becomes elevated and more opaque while the periphery remains thin and transparent.



- In primary artificial culture , meningococcus dies with in 2-3 days, probably due to the formation of **autolysin** by the organisms
- It is killed in a short time by drying and by exposure to dilute disinfectants
- It dies with in few days at zero C



Biochemical reaction

- The meningococcus is not an active fermenter and ferments glucose and maltose with the production of acid and no gas, oxidase positive and catalase positive.

Antigenic structure

- It is divided into A , B , C , D , X , Y , Z ,29E and W135 serogroups, Organisms in groups A , B and C are responsible for the great a majority of clinically recognized disease.



Virulence factors

- **Polysaccharide capsule**
- **Lipopolysaccharide (LPS) (antiphagocytic)**
- **IgA protease cleavage IgA antibodies in respiratory mucosa**



Pathogenesis

- The meningococci are **strict parasites** of man
- Air born droplet, Colonize the **nasopharynx** and from the nasopharynx enter **blood stream** and spread to **meninges** and grow in
- This bacteria are actively **toxigenic** and potent products have been obtained from cultures
- An endotoxin released by the autolysis of the bacteria is responsible for many signs of the disease



The two types of meningococcal disease

- **N. Meningitidis is the most commune cause of meningitis in person between age 2-18 years**
- **Outbreak common of meningitidis in winter and early spring**
 - 1. Cerebrospinal meningitis**
 - 2. Meningococemia meningococcal septicemia are multiplication of bacteria in the blood stream**



Laboratory diagnosis

1- Direct evidence by isolation of organisms

2- Indirect evidence by the identification of the specific antibodies

➤ which is not an important method for diagnosis of meningococcal infection

➤ Besides meningococcus other common bacteria producing pyogenic meningitis are pneumococcus and *haemophilus influenzae*

The material for the demonstration of these organisms may be:

1- Cerebrospinal fluid

2- Blood

3- Skin lesions

4- Nasopharyngeal swabs



1- Cerebrospinal fluid

This is collected by lumbar puncture under aseptic precaution it may be divided in two 3 portion:

- One for physical and cytological**
- The second for biochemical**
- The third for bacteriological examinations.**



The C.S.F is subjected to the following examinations

A- physical examinations:

- **C.S.F is clear but it became turbid if infection is present**
- **Presence of blood indicates bleeding**

B- Cytological Examination:

- **The total number of leucocytes is markedly increased and may account for thousand or more polymorphonuclear leucocytes per cram**
- **Leishman stain of the smear will reveal neutrophils**



C- Bacteriological examination

- **Smear examination**
- **Culture**
- **Oxidase test**
- **Fermentation test**
- **Latex agglutination test**
- **immunofluorescent technique**



2- Blood culture

- The importance of blood cultures in the diagnosis of meningococcal septicaemia is obvious.
- In meningococcal meningitis, blood culture is **positive in a proximately 50%** of cases, If the culture is taken **early** in the course of the disease.
- 5-10m1 blood is added to 100m1 of **tryptose phosphate broth** or **casein hydrolysate broth** , then incubated at 37C under 5-10% CO₂ .
- Subcultures are made every 48 hrs and continue for 8 days before discarded as negative.



C.S.F in meningitis

test	Normal	Acute Bacterial meningitis	Tuberculous meningitis	Aseptic meningitis
appearance	Clear Colorless	Turbid or Purulent	Clear or opalescent	clear
Total proteins	15-40ma/dl	Markedly ↑	moderately ↑	slightly ↑
sugar	50-70mg/di	markedly ↓	↓	normal
Chloride	700-740mg/d	↓	↓	normal
Cell count	0-3 lymphocyte/c mm	↑ Polymorph- nuclears	↑ Lymphocytes A few polymorphs.	↑ Lymphocytes
culture	Sterile			



Treatment and prevention

- **Penicillin G or sulfonamides are the drug of choice**
- **Third generation cephalosporin or chloramphenicol recommended for patient with allergy for penicillin**
- **meningococcal vaccine of capsular polysaccharide is available**

