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Lecture. 5
Two stage

College of Technology & Health Sciences
Department of Medical Laboratory Technique

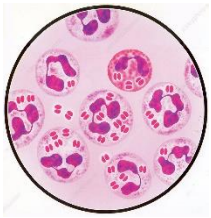
NEISSERIA

Neisseria

- Gram-negative cocci, occur in pairs (diplococci).

Neisseria gonorrhoeae (**gonococci**) and *Neisseria meningitidis* (**meningococci**) are exclusively pathogenic for humans and typically are found associated with or inside polymorphonuclear cells (PMNs).

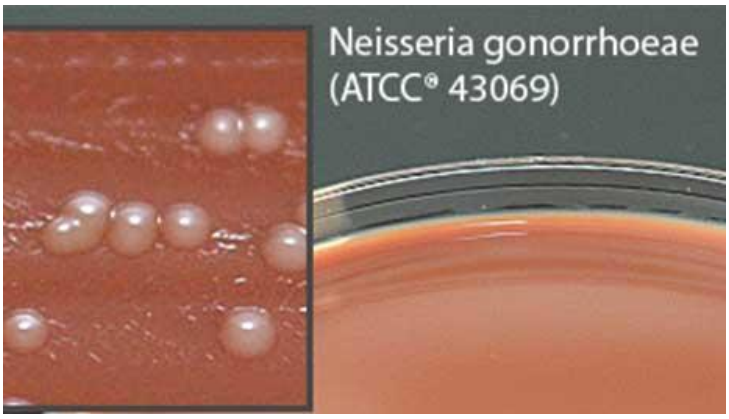
Morphology and Identification



A. Typical Organisms: Neisseria is (aerobic, Gram-negative, nonmotile diplococcus, approximately 0.8 µm in diameter. Individual cocci are **kidney bean** shaped; when the organisms occur in pairs, the flat or **concave sides** are **adjacent**.

B. Culture: -grow on **sheep blood agar**, **chocolate agar**, and **selective agar media** (eg, modified Thayer-Martin agar, Martin-Lewis agar and New York City medium).

N. meningitis grows on **sheep blood agar** as well as selective media.



N. gonorrhoeae **requires enriched chocolate** agar and/or selective media for optimal growth.

The selective media contain:

-vancomycin (suppression of Gram-positive bacteria).

- **colistin** (suppression of Gram-negative bacteria).

NEISSERIA

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other **inhibitory** substances to suppress the growth of many of the commensal microorganisms from these clinical sites.

(*N. gonorrhoeae*, *N. meningitides*, and *N. lactamica* are **colistin-resistant**, and are therefore able to grow on these selective media).

C. Growth Characteristics: The neisseriae grow best under **aerobic** conditions; however, some Neisseria species (eg, *N. gonorrhoeae*) are capable of growing under **anaerobic** conditions as well. The neisseriae produce **acid** but **not gas** by **oxidation** of various carbohydrates (not by fermentation!); the **oxidase** test is hence a key test for identifying neisseriae. Furthermore, all Neisseria species, with the exception of *N. elongata*, are **catalase positive**.

Neisseria species are grow **best** on media containing complex organic substances, such as **heated blood**, **hemin**, and **animal proteins**, and in an atmosphere containing **5%** **CO_2**. These organisms are also rapidly killed by **drying**, prolonged exposure to **sunlight**, **moist heat**, and many **disinfectants**. They produce **autolytic enzymes** that result in rapid **swelling** and **lysis** in vitro at **25**°C and at an **alkaline** pH.

NEISSERIA GONORRHOEAE Gonococci oxidize only **glucose** and differ antigenically from the other neisseriae.

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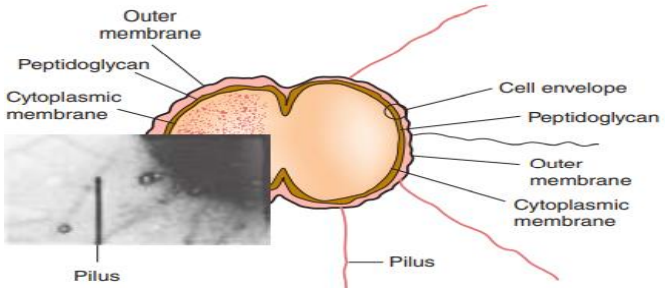
Antigenic Structure *N. gonorrhoeae* is antigenically heterogeneous and **capable** of **changing** its surface structures in vitro—and presumably in vivo—to avoid host **defenses**. Surface structures include the following.



A. **Pili (Fimbriae):** Pili are the hairlike appendages. They **enhance attachment** to host cells and **resistance** to phagocytosis. They are made up of stacked **pilin** proteins

B. **Por:** Por protein extends through the gonococcal cell membrane. It forms **pores** in the surface through which some **nutrients enter** the cell. Por proteins may impact **intracellular** killing of gonococci **within** neutrophils by **preventing** **phagosome–lysosome fusion**.

C. **Opa Proteins: adhesion** of gonococci within **colonies** and in **attachment** of gonococci to **host cell receptors**.

D. **Rmp (Protein III): is a** reduction-modifiable protein (Rmp) and changes its apparent MW when in a **reduced state**. It **associates** with Por in the formation of pores in the cell surface.

E. **Lipooligosaccharide:** In contrast to the enteric Gram-negative rods, gonococcal lipopolysaccharide (LPS) does not have long O-antigen side chains and is called a lipooligosaccharide (LOS). **Toxicity** in gonococcal infections is largely attributable to the **endotoxic** effects of LOS.

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F. Other Proteins: Lip (H8) is a surface exposed protein that is heat modifiable like Opa. The Fbp (ferric-binding protein).

Pathogenesis, Pathology, and Clinical Findings

Gonococci that form **opaque colonies** are isolated from **men** with **symptomatic urethritis** and from **uterine cervical** cultures at **midcycle**. Gonococci that form **transparent** colonies are frequently isolated from **men** with **asymptomatic urethral** infection, from **menstruating** women, and from patients with **invasive** forms of **gonorrhea**, including **salpingitis** and **disseminated infection**.

Gonococci attack **mucous membranes** of the genitourinary tract, eye, rectum, and throat, producing **acute suppuration** that may lead to **tissue invasion**; this is followed by **chronic inflammation** and **fibrosis**.

Men usually have **urethritis**, with yellow, creamy pus and painful urination. Gonococcal **bacteremia** leads to skin lesions (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists.

Gonococci can be cultured from blood or joint fluid of only 30% of patients with **gonococcal arthritis**.

Gonococcal **endocarditis** is an uncommon but severe infection.

Gonococcal ophthalmia neonatorum, an infection of the eye in newborns, is acquired during passage through an infected birth canal.

Diagnostic Laboratory Tests

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A. Specimens: Pus and **secretions** are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear. **Blood** culture is necessary in systemic illness.

B. Smears: Gram-stained smears of urethral or endocervical exudates typically reveal many diplococci within PMNs, therefore providing a presumptive diagnosis.

C. Culture Immediately after collection, pus or mucus is streaked on enriched selective medium (eg, modified Thayer-Martin medium [MTM]) and incubated in an atmosphere containing 5% CO_2 at 37°C. To avoid overgrowth by contaminants, selective media contain antimicrobial drugs (eg, vancomycin, colistin, nystatin, and trimethoprim). If immediate incubation is not possible, the specimen should be placed in a CO_2 - containing transport-culture system. Forty-eight hours after culture, identified presumptive identification can be achieved by the organisms' appearance on a Gram-stained smear and by a positive oxidase test. The definitive species level of the sub-cultured bacteria may be determined by their ability to produce acid from certain carbohydrates by oxidation; the only carbohydrate used by *N. gonorrhoeae* is glucose

NEISSERIA MENINGITIDIS

Antigenic Structure

Capsular polysaccharides: The six most important serogroups associated with disease in humans, worldwide, are A, B, C, X, Y, and W-135. Incorporation of human sialic acid derivatives such as NANA into the meningococcal capsules

allows the organism to be overlooked by the host immune system (often referred to as "molecular mimicry").

The outer membrane of *N. meningitidis* consists of proteins and LPS that play major roles in organism virulence. There are two porin proteins (Por A and Por B), interact with host cells.

The opacity proteins (Opa) are comparable to Opa of the gonococci and play a role in attachment. Meningococci are piliated and these structures initiate binding to nasopharyngeal epithelial cells and other host cells such as endothelium and erythrocytes. The lipid A disaccharide of meningococcal LPS is responsible for many of the toxic effects found in meningococcal disease. The highest levels of endotoxin.

Pathogenesis, Pathology, and Clinical Findings:

The **nasopharynx** is the portal of entry. There, the organisms **attach** to epithelial cells with the aid of **pili**; they may form part of the transient microbiota without producing symptoms and/or disease. **Invasive meningococcal diasease** (IMD) occurs in only a small number of individuals who acquired the organism and are transient carriers; infants and adolescents have the highest incidence of IMD in developed countries. From the **nasopharynx**, organisms may reach the **bloodstream**, producing meningococcal **bacteremia**; the initial symptoms during this stage of the actual infection may be similar to those of an upper respiratory tract, "flu-like" infection, but IMD quickly ensues. IMD typically presents as **meningitis**, **sepsis** (ie, meningococcemia), or as a combination of both. **Meningitis** is the most

common complication of menigococcal bacteremia. It usually begins suddenly with an **intense headache**, **vomiting**, **photophobia**, **confusion**, and **stiff neck**; it may progress to **coma** within a few hours. Fulminant meningococcemia is more severe, presenting with a high **fever** and a **hemorrhagic rash**; the patient may also develop disseminated intravascular coagulation and ultimate circulatory collapse with bilateral **hemorrhagic necrosis** of the adrenal glands with subsequent adrenal failure (Waterhouse-Friderichsen syndrome). In meningitis, the meninges are acutely inflamed, with thrombosis of blood vessels and exudation of polymorphonuclear leukocytes, so that the surface of the brain is covered with a thick purulent exudate. The exact mechanisms that transform an asymptomatic colonization of the nasopharynx into meningococcal bacteremia, subsequently leading to meningococcemia and meningitis, are not very well understood.

Diagnostic Laboratory Tests

A. Specimens: The typical specimens for isolation of *N. meningitides* include blood for culture and cerebrospinal fluid (CSF) for smear and culture. Puncture material or biopsies from petechiae may be taken for smear and culture. Nasopharyngeal swab cultures are suitable for carrier surveys.

B. Smears: Gram-stained smears of the sediment of centrifuged spinal fluid or of petechial aspirate often show typical neisseriae within polymorphonuclear leukocytes or extracellularly.

C. Culture: Although neisseriae are inhibited by certain **toxic factors** present in media and **polyanethole sulfonate** (anticoagulant) present in commercial blood

culture broths, this seems to be of a lesser problem for the ability to recover *N. meningitis* from blood cultures, compared to *N. gonorrhoeae*. CSF specimens are plated on sheep blood agar and chocolate agar and then incubated at 37°C in an atmosphere of 5% CO_2. A MTM agar favors the growth of neisseriae, inhibits many other bacteria, and is used for nasopharyngeal cultures. Colonies of *N. meningitidis* are gray, convex, and glistening, with entire edges; a positive oxidase test together with a Gram-stain showing Gram-negative diplococci provides presumptive organism identification. Spinal fluid and blood generally typically yield pure cultures that can be further identified by carbohydrate oxidative reactions and subsequent agglutination with type-specific or polyvalent serum.

D. Serology: Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests or by their bactericidal activity.