

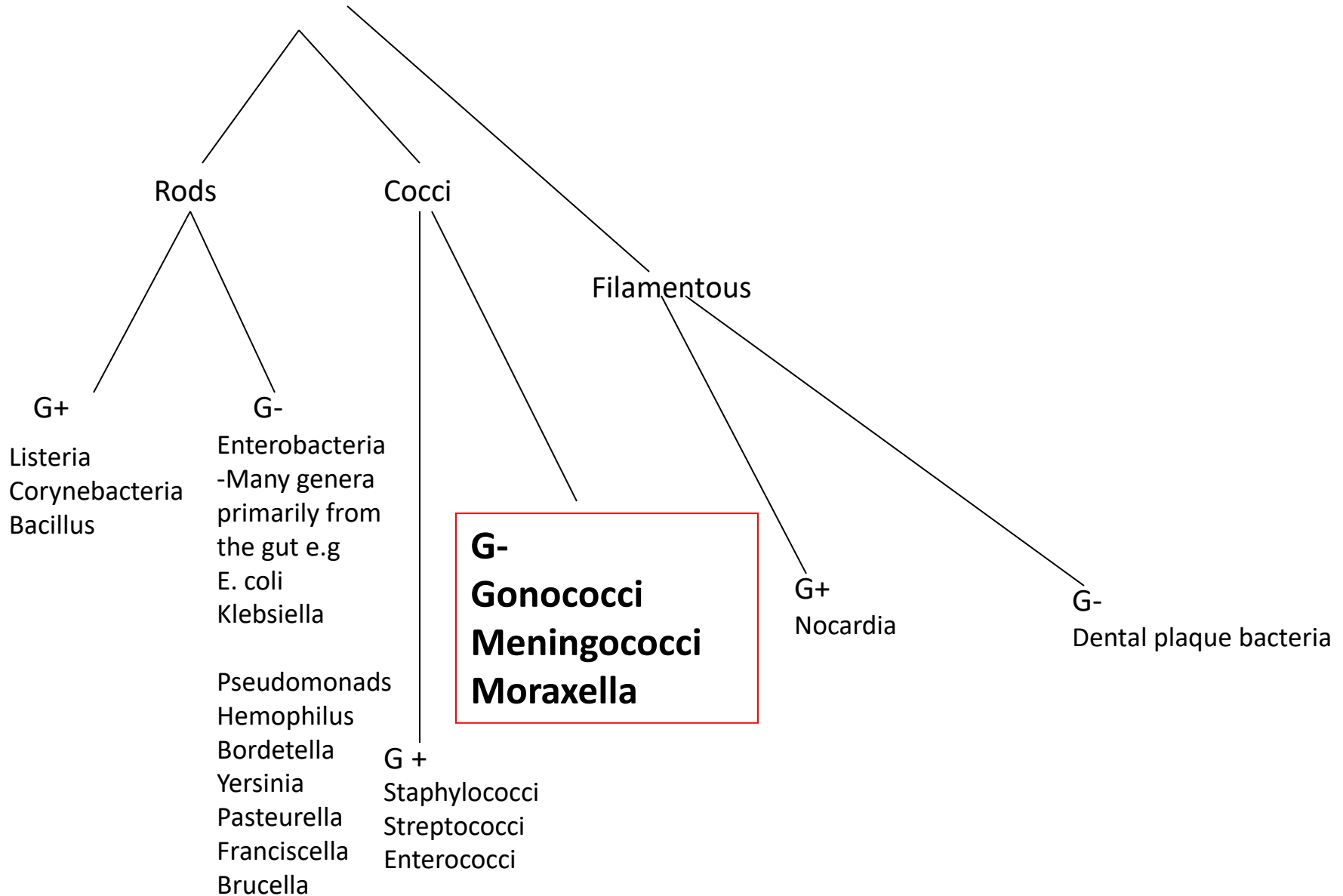


# Gram Negative Cocci

By

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# Aerobes (Common)



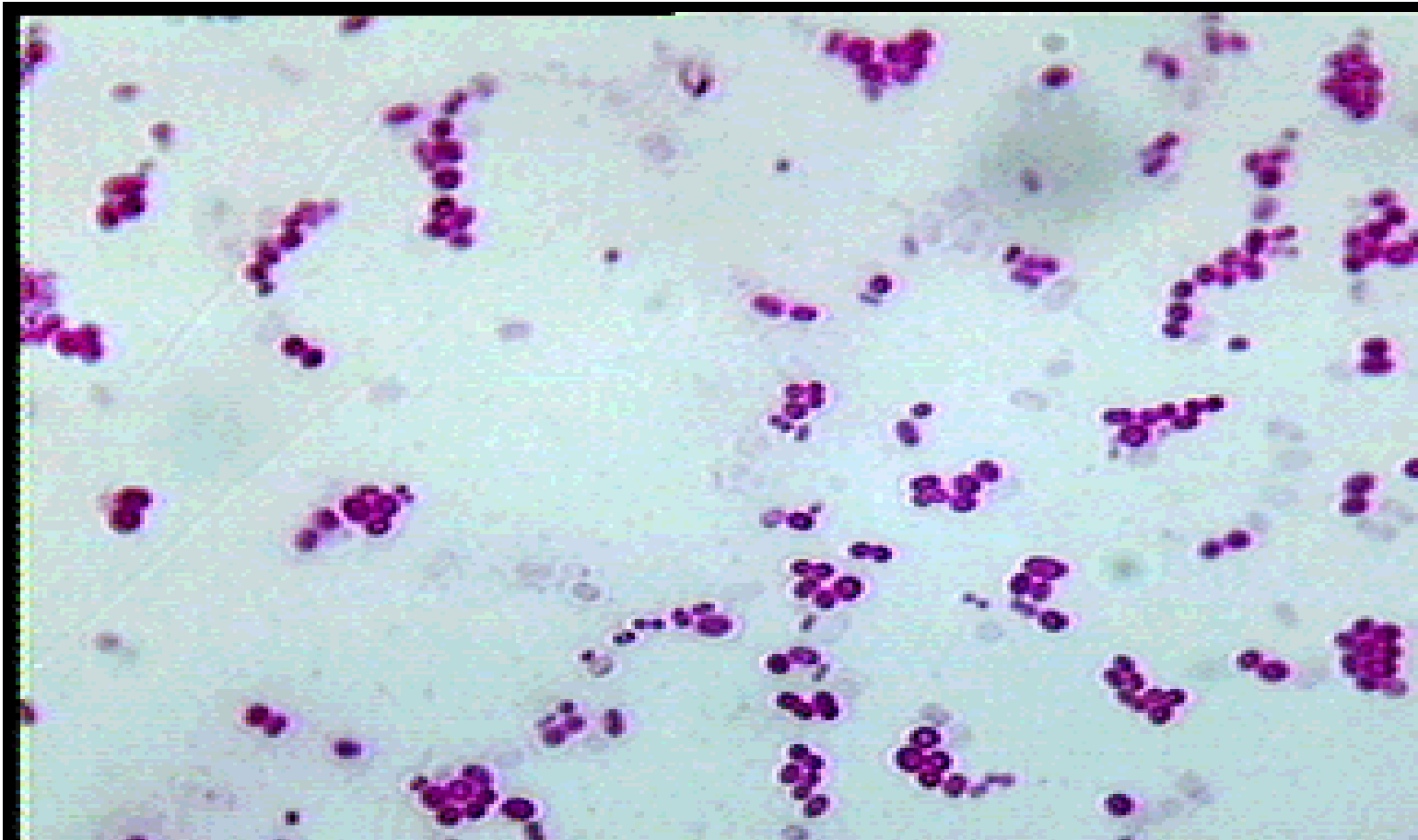
# Neisseria: Gram negative cocci

- Very few G- cocci
  - Some *Neisseria*, *Moraxella* are normal microbiol flora
  - Of greatest interest: these pathogenic ones
- *N. meningitidis* and *N. gonorrhoeae*.
  - Delicate and fastidious;
  - oxidase +, coffee bean shaped in pairs
  - pyogenic

# The Neisseriae

- Gram – cocci; usually in pairs, sometimes piliated. 2 major species: gonococci and meningococci – differentiated by the usual clinical presentations of the diseases they cause.
- Cultures: after 48h on enriched media (Mueller-Hinton) form convex, glistening, elevated, mucoid colonies, 1-5mm; non-haemolytic, transparent colonies.
- Produce oxidase. Test: paper soaked with tetramethyl-paraphenylenediamine hydrochloride → dark purple rapidly.
- Rapidly killed by drying, sunlight, moist, heat and many disinfectants.

# NEISSERIA: DIPLOCOCCI



## *Neisseria gonorrhoeae*

- *N. gonorrhoeae*: cause of gonorrhea, an STD
  - Virulence factors: capsule, fimbriae, survival in neutrophils
  - Males: painful purulent discharge from urethra
  - Females: often asymptomatic; otherwise, urethritis, PID, infections anywhere sexual contact occurs, arthritis.
  - Eye infection of newborns – medical emergency
  - No long term immunity!

Pelvic inflammatory disease (PID): infection and inflammation of the upper genital tract in women. Affects the uterus, fallopian tubes, ovaries. Scarring can lead to infertility, tubal (ectopic) pregnancy, chronic pelvic pain, abscesses, and other serious problems. PID is the most common preventable cause of infertility.

# *N. gonorrhoeae*: structures and function

- Opa proteins
- Pili (fimbriae)
- Por
- Lipooligosaccharide
- Rmp (protein III)
- Other proteins

# *N. gonorrhoeae*: structures and function

## **Opa (protein II)**

A specific strain of gonococcus can express 1, 2, sometimes 3 types of Opa protein.

Some may not express Opa at all .

Each strain has 11 or 12 genes for different Opa.

- They have 2 main functions
  1. Attachment of gonococci to host cell receptors
  2. Adhesion of gonococci within colonies
- One portion of the Opa molecule is in the outer membrane of the gonococcus and the rest is exposed on the surface
- Molecular weight : 20-28 kDa



# *N. gonorrhoeae*: structures and function

## **Pili**

Found on the surface of the gonococcus – another name is fimbriae .  
These are hair like appendages which extend several micrometers.

Pili are made up of pilin proteins stacked together.

Pilins of nearly all strains of *N. gonorrhoeae* are antigenically different.

The 3 parts of the pilin molecule:

Amino terminal is conserved

Amino acid sequence of the middle portion is also conserved; serves in attachment to host cell but less so in the immune response.

Amino acid sequence near the carboxyl terminal is highly variable; being most prominent in the immune response.

- They have 2 main functions
  1. Enhance attachment to host cells
  2. Enhance resistance to phagocytosis

# *N. gonorrhoeae*: structures and function

## **Por**

This protein extends through the gonococcal cell membrane. Its occurrence in trimers form pores in the surface through which some nutrients enter the cell. Molecular weight is 32 – 36 kDa.

Por proteins may impact intracellular killing of gonococci within neutrophils by preventing phagosome-lysosome fusion.

Variable resistance of gonococci to killing by normal human serum depends on whether Por protein selectively binds to complement components C3b and C4b.

Each strain of gonococcus expresses only one of two types of Por but the Por of different strains is antigenically different.

# *N. gonorrhoeae*: structures and function

## **Lipooligosaccharide (LOS)**

LOS is a modified lipopolysaccharide (which is what gram negative rods have) but without the long O-antigen side chains.

Gonococci can express more than one antigenically different LOS chain simultaneously.

LOS is responsible for toxicity in gonococcal infections through its endotoxic effects.

Eg LOS causes ciliary loss and mucosal cell death in fallopian tubes

Also LOS through the activity of sialyltransferase makes gonococci resistant to killing by the human antibody-complement system and interferes with gonococcal binding to receptors on phagocytic cells.

# *N. gonorrhoeae*: structures and function

## **Rmp (protein III)**

This is referred to as reduction-modifiable protein.

Molecular weight is 30-31 kDa.

It changes its apparent molecular weight when in a reduced state (by loss of hydrogen ion).

Rmp is antigenically conserved in all gonococci.

It associates with Por in the formation of pores in the cell surface.

**Other proteins:** these have poorly defined roles

**Fbp**-ferric binding protein

**Lip (H8)**; a surface exposed protein

**IgA1 protease**, similar to that found in meningococci, *H. influenzae* and strept pneumo.

# Heterogeneity of *N. gonorrhoeae*

Gonococci exhibit genetic and antigenic heterogeneity.

They have evolved mechanisms for frequently switching from one antigenic form (Opa, pilin or LOS) to another.

This takes place in one in every  $10^{2.5} - 10^3$  gonococci.

The molecules rapidly switching from one antigenic form to another helps the gonococci elude the host immune response.

Gonococci contain several plasmids, which are transmissible by conjugation.

Some plasmids that code for  $\beta$ -lactamases have been found in gonococci but are derived from *Haemophilus* and other gram negative organisms.

# Neisseria gonorrhoeae

- MOT: Sexual contact
- Men: acute urethritis
  - painful urination
  - yellowish penile discharge
  - “the drip”
- Women: mostly asymptomatic
  - more complications:PID, infertility
- Babies: ophthalmia neonatorum

# *Neisseria gonorrhoeae*

- Pathogenesis: gonococci attack mucous membranes of GUT, eyes, rectum:
- Acute suppuration → tissue invasion → chronic inflammation → fibrosis; spread to other tissues.
- Gonococcal bacteraemia leads to skin lesions, haemorrhagic papules and pustules.
- When the suppuration occurs in the eye of the newborn – gonococcal ophthalmia neonatum.

# *Neisseria gonorrhoeae*

- Rx: localised infection – serum-sensitive (killed by Abs and c') bacteria; whereas bacteraemia – serum-resistant.
- Diagnostic lab tests: taken from pus & secretions from urethra, cervix, throat, synovial fluid and smears from endocervix and urethral are stained.
- Disease: worldwide, sexually, multiple sexual partners, asymptomatic infection.



# *Neisseria gonorrhoeae*

- Global estimated incidence of gonorrhoea is 62 million people annually.
- Third most commonly sexually transmitted disease in USA
- Microbial physiology and structure
  - Morphology
    - Cell—small Gram<sup>-</sup> diplococci
    - Colony—grow on chocolate agar, Thayer-Martin agar, and Martin-Lewis agar
    - Inhibited by fatty acids and trace metals

# *Neisseria gonorrhoeae*

- Physiology
  - Susceptible to desiccation—require 5% CO<sub>2</sub>
  - Colony types (T1-T5) -based on presence of pili and virulence
    - T1 and T2 virulent; others are not
    - Identification:
      - Colony morphology
      - Presence of cytochrome oxidase
      - Strict oxidative metabolism of glucose, but not other carbohydrates

# *Neisseria gonorrhoeae*

- Pathogenesis
  - Encounter and entry
    - Only present in humans; not present in environment or other animals – proven in several animal experiments
    - Both men and women can be asymptomatic (normally women)—important to trace and treat contacts
      - Doesn't colonize vagina in post-pubescent girls
        - » Attaches to columnar epithelium not squamous epithelium
    - Can colonize throat and rectum

# *Neisseria gonorrhoeae*

- Pathogenesis

- Process

- Introduced into vagina or urethral mucosa of penis—attach to surface of epithelial cells and multiply
      - Attach to unidentified specific receptors by pili (also prevent phagocytosis)
      - Protein II—once cells brought close enough by pili, adherence to cell occurs (also called OPA)

# *Neisseria gonorrhoeae*

- Pathogenesis
  - Process
    - Localization due to method of introduction, not tissue preference
      - Gonococcal pharyngitis (oral-genital sex)
      - Gonococcal proctitis (anal intercourse)

# *Neisseria gonorrhoeae*

- Spread and multiplication
  - Once attach to non-ciliated cells, multiply rapidly and spread up urethra (male) or through cervix (female)
    - Are not motile; aided external mechanisms
      - Eddy current in mucus
      - Attached to spermatozoa
    - Protected from IgA by IgA protease

# *Neisseria gonorrhoeae*

- Spread and multiplication
  - Invasion
    - Once attach, microvilli extend and embrace organisms
    - Internalized by endocytosis into “nonprofessional phagocytes” and transported in cell by phagocytic vesicles
      - Coalesce to form larger vesicles—gonococci multiply
      - Sheltered from Ab and professional phagocytes

# *Neisseria gonorrhoeae*

- Spread and multiplication
  - Invasion
    - After internalization, transported to base of non-ciliated cells
      - Vacuoles fuse with basement membrane and discharge contents into subepithelial connective tissue
      - Either cause local damage or enter blood vessels and cause disseminated disease



# *Neisseria gonorrhoeae*

- Survival in bloodstream
  - Most strains killed by antibodies
    - Target surface proteins and LPS
  - Resistant strains cause disseminated disease
    - Distinguishing characteristics
      - More sensitive to penicillin
      - Have specific nutritional requirements
      - Attachment sialic acid to LOS; blocks complement of activation and binding to other surface proteins
  - Women tend to have disseminated disease affecting joints
    - Toxic products spread in blood from local site

# *Neisseria gonorrhoeae*

- Damage
  - Ciliated cells—done by endotoxin and fragments of murein (muramyl monomers, same as *Bordetella pertussis*)
    - Studied most in fallopian organ culture
    - Ciliary mechanism important for transporting egg from ovary to uterus and for clearing bacteria from mucosal surface
      - After damage, fallopian tube susceptible to bacteria from vaginal flora that ascend through the cervix and uterus

# *Neisseria gonorrhoeae*

- Damage
  - Submucosal connective tissue, due to inflammatory response
    - Male urethra—leads to local symptoms (pain on urination and pus discharge)
      - Other diseases cause by other pathogens have some of the same symptoms
      - Pain is more intense and urethral discharge is more copious, thicker, and greenish-yellow

# *Neisseria gonorrhoeae*

- Outcome of infection
  - Males—symptoms usually subside in several weeks, even without treatment
    - Repeated infections, in untreated, can lead to scarring and stricture of urethra
      - Relatively unusual, because males usually seek treatment
  - Epididymis can become involved=>epididymitis

# *Neisseria gonorrhoeae*

- Outcome of infection
  - Females—local urogenital infection frequently asymptomatic
    - Complications yield symptoms
    - Sequellae of fallopian tube damage
      - Ectopic pregnancy, recurrent PID by other organisms, chronic pelvic pain, and infertility due to blockage or damage to tubes
    - Can lead to death of woman and her unborn child

# *Neisseria gonorrhoeae*

- Prompt treatment for either gender decreases chances of disseminated disease
- Epidemiology: sexual transmission
  - Males—20% after intercourse
  - Females—50% after intercourse

# *Neisseria gonorrhoeae*

- Diagnosis
  - Gram<sup>-</sup> intracellular diplococci in vaginal, cervical, or urethral specimens is suggestive; start antibiotic therapy
  - Important to culture for confirmation
  - Biochemical tests
    - Oxidase+
    - Oxidize glucose, not maltose nor sucrose

# *Neisseria gonorrhoeae*

- Gonococci can be cultured from blood or joint fluid of only 30% of patients with gonococcal arthritis.
- Sensitivity of stained smears of urethral exudate from men is 90% while specificity is 99%
- Sensitivity of stained smears of endocervical exudates from women is 50% while specificity is 95%
- Additional diagnostic testing is not required in men with positive smears but cultures or nucleic acid amplification tests should be done for women
- Generally, specimens from throat and rectum are not helpful in diagnostics



# *Neisseria gonorrhoeae*

- Treatment
  - Used to be massive doses of penicillin
  - Now: If  $\beta$ -lactamase resistant (plasmid; same as *Haemophilus influenzae*); cephalosporin, ceftriaxone-IM
  - Resistance to penicillin, tetracycline, aminoglycosides (chromosomal; change in cell surface)—susceptibility test
  - Single dose oral therapy

# *Neisseria gonorrhoeae*

- Treatment
  - Single dose oral therapy: ciprofloxacin; cefixime
    - Resistance increasing
    - Therefore not recommended
  - If complicated by *Chlamydia*: doxycycline; azithromycin

# OPHTHALMIA NEONATORUM



# Ophthalmia neonatorum

- Infection of the eye of the newborn
- Acquired during passage through an infected birth canal.
- Progress is usually rapid=>medical emergency
- If untreated, can result in blindness.
- To prevent gonococcal ophthalmia neonatorum, instillation of tetracycline, erythromycin, or silver nitrate into the conjunctival sac of the newborn is compulsory in the United States.

# Gonococcal vaccine

- Considerable antigenic variability have hampered the development of a vaccine for gonorrhoeal disease
- Pilin has been tried with only moderate success in military recruits but protection was strain limited
- Porin was also studied but the induced anti-porin antibodies were not bactericidal
- Complete genome sequence may hopefully help in identifying candidate proteins.

# Neisseria-2

- *N. meningitidis*: a cause of bacterial meningitis
  - Capsule protects cells during phagocytosis
  - Present as normal flora in 40% of population
  - Transmitted by droplets, close contact
  - Infection of meninges, sore throat, high fever, headache, stiff neck, vomiting, convulsions, petechiae; rapid.
  - Epidemiology: people living in close contact; military recruits currently vaccinated; because of outbreaks, push to vaccinate college students in developed countries.

# The Meningitis Belt



# *Neisseria meningitidis*

- At least there exists 13 serogroups, identified by immunologic specificity of capsular polysaccharides.
- The most important serogroups associated with disease in humans are A, B, C, X, Y, W-135.
- The grp A polysaccharide is a polymer of N-acetylmannosamine phosphate while that of grp C is a polymer of N-acetyl-O-acetylneuraminic acid.
- The outer membrane proteins of meningococci have been divided into classes based on molecular weight and all strains have either class 1, 2 or 3 proteins (analogous to Por proteins of gonococci) which are responsible for the serotype specificity. There are 20 serotypes defined.
- Meningococcal LPS is responsible for many of the toxic effects found in meningococcal



# *Neisseria meningitidis*

- MOT: respiratory droplets
- Causes meningococemia
  - disseminated intravascular coagulopathy
  - skin rashes → purpura
  - meningitis

# *Neisseria meningitidis*

- Morphology
  - Encapsulated Gram<sup>-</sup> diplococci
- Colonization
  - Asymptotically in nasopharynx of healthy individuals

# *Neisseria meningitidis*

- Microbial physiology and structure
  - Colony morphology
    - Form transparent, non-pigmented, non-hemolytic colonies on chocolate blood agar in 5% CO<sub>2</sub>
      - Capsule indicated by large mucoid colonies
  - Physiology
    - Oxidase+
    - Acid production from glucose and maltose, but not sucrose or lactose
    - Several serotypes known

# *Neisseria meningitidis*

- Causes of pathogenesis
  - Ability to colonize nasopharynx due to presence of pili
  - Ability to resist host defenses and spread due to presence of capsule
  - Expression of toxic effects due to presence of endotoxin

# *Neisseria meningitidis*

- Disease process
  - Attach to specific receptors on non-ciliated cells of nasopharynx
    - Pili—mutants have decreased cell binding ability
  - Organisms internalized into phagocytic vesicles by endocytosis
    - After 18-24 hours, found in sub-epithelial tissue space

# *Neisseria meningitidis*

- Tissue damage
  - Type of damage
    - Endothelial damage
    - Inflammation of blood vessel walls
    - Thrombosis
    - Disseminated intravascular coagulation
  - Mechanism (endotoxin)
    - Continuous production of excess membrane fragments that contain LPS

# *Neisseria meningitidis*

- Epidemiology
  - Transmitted by respiratory secretions to people living in close contact
  - Incidence during Spring
  - Susceptibility
    - Children <5 years old
    - Older individuals infected during epidemics
    - Maternal Ab protection for first 6 months

# *Neisseria meningitidis*

- Syndromes
  - Meningitis
    - Symptoms: headache and fever (young children—fever and vomiting)
    - Mortality
      - Untreated: 100%
      - Treated:  $\leq 15\%$  when antibiotics administered promptly
    - Incidence of neurological problems is low



# *Neisseria meningitidis*

- Syndromes
  - Meningococemia (with or without meningitis)
    - Life-threatening, with mortality of 25%, even when promptly treated
    - Pathology
      - Thrombosis of blood vessels
      - Multiorgan involvement
      - Small skin lesions on trunk and lower extremities (may form larger hemorrhagic lesions)
      - May progress to completely debilitating DIC with shock (destruction of adrenal gland)

# *Neisseria meningitidis*

- Syndromes
  - Milder septicemia (chronic)—persist for days or weeks
    - Symptoms: low-grade fever, arthritis, skin lesions
    - Response to antibiotics normally excellent

# MENINGOCOCCEMIA



# *Neisseria meningitidis*

- Laboratory diagnosis
  - Blood and cerebrospinal fluid (especially inside neutrophils)
- Treatment
  - Penicillin (resistant strains rarely seen)
  - Chloramphenicol, ceftriaxone, cefotaxime

# *Neisseria meningitidis*

- Prevention and control (high-risk groups)
  - Use of prophylactic antibiotics
    - Penicillin not effective in eliminating carrier state
    - Antibiotics of choice:
      - Sulfonamide
      - Rifampin, for sulfonamide-resistant strains
      - Sulfadiazine

# *Neisseria meningitidis*

- Prevention and control (high-risk groups)
  - Vaccines: there are 3 types of vaccines available
    - Polysaccharide vaccines have been available for >30yrs. These meningococcal vaccines are available in either bivalent (grps A & C), trivalent (grps A, C & W) or tetravalent (grps A, C, Y and W135) for control
    - Group B polysaccharide vaccines cannot be developed, due to antigenic mimicry with polysaccharide in human neurologic tissues. Consequently vaccines against B were outer membrane proteins and strain-specific to control specific epidemics. There are however grp B protein vaccines are in late stages of development
    - Since 1999 meningococcal conjugate vaccines against grp C have been available and widely used. Tetravalent A, C, Y and W135 conjugate vaccines have been licensed since 2005 for use in children in Canada, the United States of America, and Europe.

# *Neisseria meningitidis*

- Prevention and control (high-risk groups)
  - Global Health Response
    - WHO promotes a strategy comprising
      - Epidemic preparedness – focusing on surveillance, from case detection to investigation and laboratory confirmation.
      - Prevention – consists of vaccinating all 1-29 year olds in the African Meningitis belt with the new meningococcal A conjugate vaccine.
      - Response
    - Epidemic response consists of prompt and appropriate case management with oily chloramphenicol or ceftriaxone and reactive mass vaccination of populations not already protected through vaccination.

## *Other Neisseria*

- *Neisseria lactamica*
- *N. sicca*
- *N. subflava*
- *N. flavescens*
- *N. mucosa*
- *N. cinerea*



# Comparison of Meningococcus and Gonococcus

- Similarities:
  - Both diplococci
  - Share about 80% DNA
  - Neither makes exotoxin; both make endotoxin
  - Both are relatively good colonizers
    - Other species colonize mucous membranes and skin, and rarely cause disease

# Comparison of Meningococcus and Gonococcus

- Differences:
  - Gonococcus causes gonorrhoea
    - Localized inflammation (usually in urethra) and is very rarely fatal
  - Meningococcus causes meningitis and other diseases
    - Systemic and life-threatening disease

# Comparison of Meningococcus and Gonococcus

- Why are diseases so different?
- Presence in normal flora
  - 10% healthy people colonized by meningococcus in posterior pharynx
  - Gonococcus not present

# Comparison of Meningococcus and Gonococcus

- Penetration of mucous membranes
  - Mechanism similar for the two organisms
  - Penetrate different mucous membranes columnar epithelial cells
    - Meningococcus—nasopharynx
    - Gonococcus—fallopian tubes

# Comparison of Meningococcus and Gonococcus

- Survival against host defenses
  - Gonococcus killed in bloodstream
  - Meningococcus grows extremely fast in bloodstream, reaching very high blood titers
    - Reason: large capsule and outer membrane proteins

# Comparison of Meningococcus and Gonococcus

- Signs and symptoms of two diseases
  - Gonococcus (highly invasive) cause symptoms frequently, but sites are localized
    - Production of pus and pain during urination
    - Sequellae depending on location
      - Inflammation of fallopian tubes
      - Pelvic inflammatory disease
        - » May result in scarring, leading to infertility and ectopic pregnancy

# Comparison of Meningococcus and Gonococcus

- Signs and symptoms
  - Meningococcus (highly serum-resistant )- causes septicemia with multiple organ involvement, including CNS
  - Damage caused by
    - Disseminated intravascular coagulation (DIC), accompanied by shock, fever, and other responses
    - Due to large amount of endotoxin in blood, tissues
    - Due to ability of meningococcus to thrive in blood

# *Moraxella*

- Aerobic
- Gram negative cocci -
- Facultative
- Non motile
- Nonfermentative
- Oxidase-positive
  
- They form part of the normal flora of the respiratory tract
- May cause bacteraemia, endocarditis, conjunctivitis & meningitis
- Most are susceptible to penicillin



# *Taxonomy of Neisseria*

Order: Neisseriales

Family – Neisseriaceae

Genus – Neisseria

Species – Neisseria meningitidis

# *Taxonomy of Moraxella*

Family – Moraxellaceae

Genus – Moraxella (another is acinetobacter)

Subgenus: 1. Moraxella – very short and plump  
approaching coccus shape

- M. Atlantae
- M. Lacunata
- M. Nonliquefaciens
- M. osloensis

2. Branhamella – cocci shaped

- M. catarrhalis

# *Moraxella catarrhalis*

Previously called *Branhamella catarrhalis*, *Neisseria catarrhalis*, *Micrococcus catarrhalis*

Forms part of normal flora in 40-50% of healthy children

Causes the following infections:

- Bronchitis
  - Pneumonia
  - Sinusitis
  - Otitis media
  - Conjunctivitis
- 
- It is a cause of concern in immuno-compromised patients
  - Most strains of clinically significant *M catarrhalis* produce  $\beta$ -lactamase

# *Moraxella catarrhalis*

2 main characteristics differentiate *M catarrhalis* from neisseriae

- a) *M catarrhalis* lacks the ability of carbohydrate fermentation
- b) *M catarrhalis* is characterized by its production of DNase.

Similarity with neisseriae are

- Positive oxidase reaction
- Fastidious growth