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# Gram-negative cocci, *Neisseria* sp., *Haemophilus* sp.

key points of the lecture

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# genus *Neisseria*

- Growth: in a moist atmosphere containing 5–10% carbon dioxide
- Catalase positive
- Gram-negative **diplococci**, don't produce spores, non-motile
  
- Obligate human pathogens
- Common commensals of the upper respiratory tract

# Gram-negative cocci pairs (diplococci)

- Typically found inside polymorphonuclear pus cells of the inflammatory exudate

Neisseria diplococci resemble **coffee beans** when viewed microscopically

# Haemolytic activity

- Gama – no hemolysis on BA

- It requires an aerobic environment with added CO<sub>2</sub> and enriched media such as chocolate agar for growth.
  - Oxidase and catalase positive
  - Sensitive to **!drying and cold!** – collected material **cannot be placed in the refrigerator**
  - Colonies of meningococci and gonococci react quickly in the test for cytochrome oxidase; nonpathogenic neisseriae react more slowly
- 
- found as part of the normal flora of the human upper respiratory tract – nonpathogenic
  - important human pathogens

# Medical important species

*Neisseria meningitidis*

*Neisseria gonorrhoeae*

*N. lactamica*, *N. cinerea*, *N. subflava*, *N. sicca*, etc. –  
commensal species of the upper respiratory tract

# *NEISSERIA MENINGITIDIS*

- 13 serogroups, based on antigenic differences in their capsular polysaccharides
- six of these have significant pathogenic potential: Over 90% of worldwide invasive disease is caused by strains from **serogroups A, B, C, W-135, X and Y.**
- Geographic distribution and epidemic potential differ according to serogroup.

# Pathogenesis

- The natural habitat of the meningococcus is the human nasopharynx
- transmission is largely via close contact (intimate, kissing)
- **The bacteria are transmitted from person-to-person** through droplets of respiratory or throat secretions from carriers.
- Around 5–10% of general populations are normally carriers of meningococci.
  - ⊙ Close and prolonged contact – such as kissing, sneezing or coughing on someone, or living in close quarters with an infected person (a carrier) – facilitates the spread of the disease.
  - ⊙ The average incubation period is 4 days, but can range between 2 and 10 days.
  - ⊙ only infects humans; there is no animal reservoir.



# *Neisseria meningitidis*

- The most common symptoms are a **stiff neck, high fever, sensitivity to light, confusion, headaches and vomiting.**

# *Neisseria meningitidis*

- A less common but even **more severe** (often fatal) form of meningococcal disease is **meningococcal septicaemia**, which is characterized by a haemorrhagic rash and rapid circulatory collapse.
- During meningococcal septicaemia there are signs and symptoms of circulatory failure, multi-organ dysfunction and coagulopathy.

# Laboratory diagnosis

- Sampling
  - throat swabs, pus, blood cultures, cerebrospinal fluid (CSF)
- Microscopy
- Cultivation on enriched BA, chocolate agar overnight at 37°C in an atmosphere of 5–10% carbon dioxide
- A rapidly positive oxidase test
- A rapidly positive catalase test
- Sugar utilization tests (G, M)
- Meningococcal capsular polysaccharide may be detected in CSF by latex agglutination.

# *Neisseria meningitidis*

- ⦿ a lumbar puncture showing a purulent spinal fluid.
- ⦿ The bacteria can sometimes be seen in microscopic examinations of the spinal fluid.
- ⦿ The diagnosis is supported or confirmed by growing the bacteria from specimens of spinal fluid or blood, by agglutination tests or by polymerase chain reaction (PCR).
- ⦿ The identification of the serogroups and susceptibility testing to antibiotics are important to define control measures.

## ATB susceptibility *Neisseria meningitidis*

- Intravenous penicillin, cefotaxime or ceftriaxone are the drugs of choice.

# Chemoprophylaxis & vaccination

- All household and other intimate contacts of a case should be given chemoprophylaxis to eliminate carriage.
- Rifampicin is the preferred drug for children, although resistance can develop rapidly.
- Ciprofloxacin is used widely for adolescents and adults as a single oral dose.
- Vaccination - vaccines containing the native, unconjugated, group-specific capsular polysaccharide of meningococci of groups A, C, Y and W-135 are available

# *NEISSERIA GONORRHOEAE*

- ◎ The name 'gonorrhoea' derives from the Greek words *gonos* (seed) and *rhoia* (flow), and described a condition in which semen flowed from the male organ without erection.
- ◎ gonorrhoea is associated with sexual promiscuity

# *NEISSERIA GONORRHOEAE*

◎ gonorrhoea is a classical venereal disease:

- being spread almost exclusively by sexual contact,
  - having a short incubation period and
  - being relatively easy to diagnose and treat
- 
- Also cause: conjunctivitis, urethritis, prostatitis and orchitis



# Pathogenesis

- *N. gonorrhoeae* is exclusively a human pathogen, although chimpanzees have been infected artificially
- It is never found as a normal commensal
- A proportion of those infected, particularly women, may remain asymptomatic
- The areas most frequently involved are the cervix, urethra, rectum, pharynx and conjunctiva
- Gonorrhoea in young girls may present as vulvovaginitis

# Symptoms:

- Purulent discharge from genitals (often with smell)
- Inflammation
- Redness
- Swelling
- Dysuria
- Burning sensation when urinating

# ophthalmia neonatorum

- Babies born to infected women may suffer ophthalmia neonatorum, in which the eyes are coated with gonococci as the baby passes down the birth canal.
- A severe purulent eye discharge with peri-orbital oedema occurs within a few days of birth.
- If untreated, ophthalmia leads rapidly to blindness.
- Prevention

◎ Swab from vagina or discharge

- on blood agar,
- modified blood agar,
- chocolate agar + ATB – inhibition of contaminating flora

◎ Grey colonies

◎ after application of cytochromoxidase – become black

◎ – biochemical tests for diff.dg. from other *Neisseria*

# NEISSERIA tests

## *Neisseria gonorrhoeae*

- Glucose: Positive
- Maltose: Negative
- Fructose: Negative
- Sucrose: Negative

## *Neisseria meningitidis*

- Glucose: Positive
- Maltose: Positive
- Fructose: Negative
- Sucrose: Negative

# ATB susceptibility *Neisseria gonorrhoe*

- Penicillin
- Ceftriaxone or cefixime
- ciprofloxacin,  
azithromycin,  
tetracyclines

# *Moraxellae*

- non-fermentative organism that may be coccoid.
- The most important member of the group, *Moraxella catarrhalis* (formerly known as *Branhamella catarrhalis*)
  - a common commensal of the upper respiratory tract and
  - an opportunistic pathogen associated with otitis media in children and exacerbations of chronic obstructive pulmonary disease in adults.

# Key points *Neisseria*

- *Neisseria meningitidis* (meningococcus) and *N.gonorrhoeae* (gonococcus) are obligate human parasites



# Key points *Neisseria meningitidis*

- commensally in the nasopharynx,
- transmitted via close kissing contact
- disease varies in severity from mild sore throat to meningitis, septicaemia or septicaemic shock
- Treatment - intravenous administration of antibiotics.
- Prophylactic antibiotics can be given to contacts to eradicate carriage and control outbreaks
- Of the 13 serogroups, groups A, B, C, W-135, X and Y cause more than 90% of cases. Vaccines are available against A, C, W-135 and Y

# Key points *Neisseria gonorrhoeae* & *Moraxella*

- *N. gonorrhoeae* causes the sexually transmitted disease gonorrhoea. Asymptomatic carriage in women is common, but the organism may give rise to acute salpingitis, which may be followed by pelvic inflammatory disease and a high probability of sterility if inadequately treated
- cephalosporins such as ceftriaxone are the drugs of choice
- Early diagnosis, effective treatment and contact tracing are key to preventing the spread of disease. There is no effective vaccine to prevent gonorrhoea.
- *Moraxella catarrhalis* is an upper respiratory tract commensal that causes lower respiratory tract infections and otitis media

# *Haemophili*

- Small
- pleomorphic
- Gram-negative rods or coccobacilli with occasional longer, filamentous forms
- catalase and oxidase positive

# Growth requirements

- **X factor** (haemin) –
  - required for the synthesis of cytochrome c and other iron-containing respiratory enzymes.
- **V factor** is nicotinamide adenine dinucleotide (NAD) –
  - essential for oxidation-reduction processes in cell metabolism.
- *H. influenzae* requires both factors
- *H. parainfluenzae* only V factor

# Virulence factors

- Capsule - antiphagocytic
- Fimbriae – attachment to epithelial cells
- IgA proteases – involved in colonization
- Outer membrane proteins and lipopolysaccharides – involved in invasion

# Medical important species

*Haemophilus influenzae*

*Haemophilus parainfluenzae*

*Haemophilus ducrey*

# *HAEMOPHILUS INFLUENZAE*

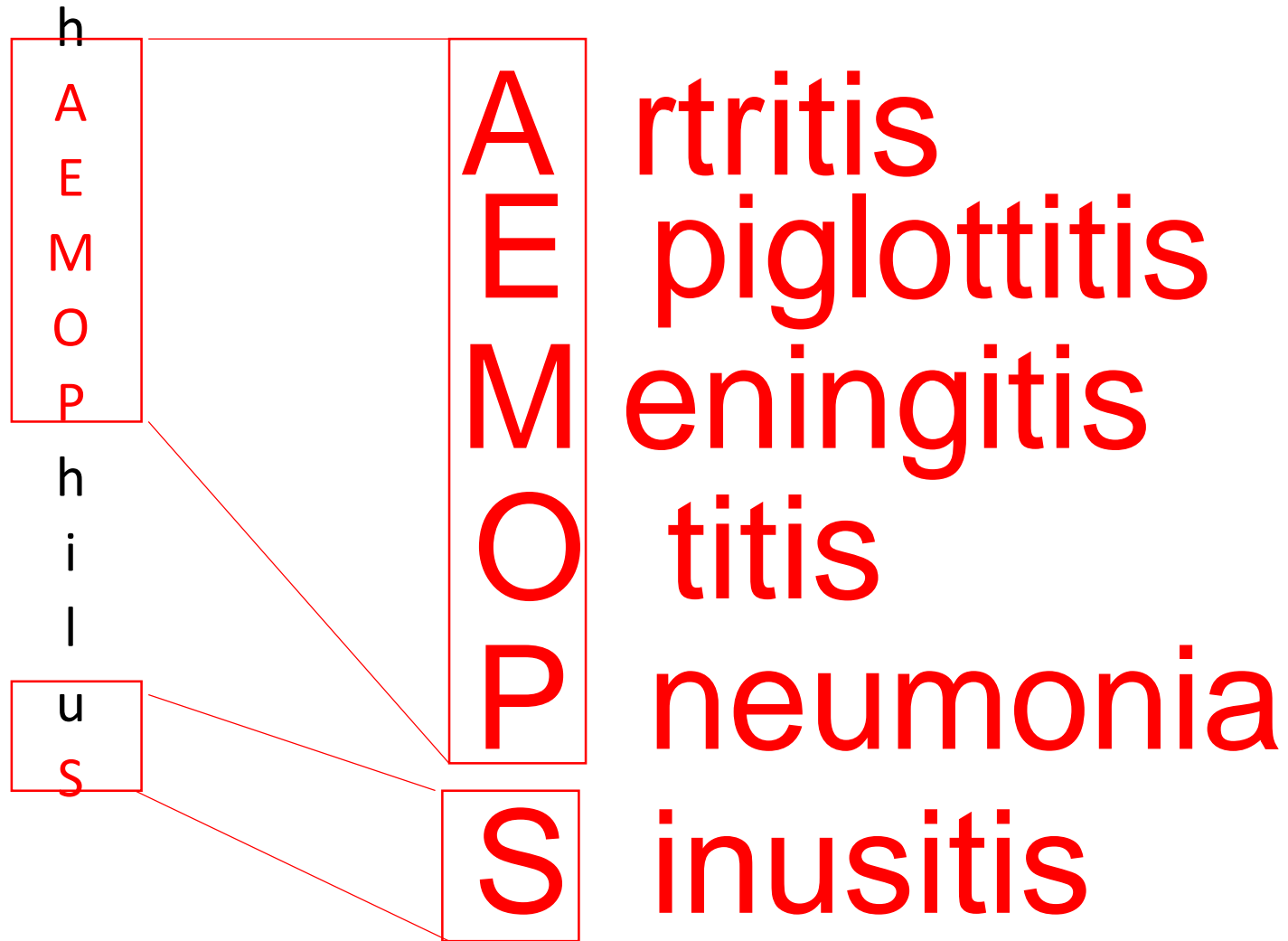
- 6 capsular types, designated a–f, which can be identified by a polymerase chain reaction (PCR) method.
- The most important is **type b**
- The polysaccharide capsule is the major virulence factor for Hib.
- When the organism invades the bloodstream, the capsule enables the organisms to evade phagocytosis and complement-mediated lysis in the non-immune host.

# *Haemophilus* infections

- **Noninvasive** – upper respiratory tract infection or superinfection (overinfection) or normal flora of URT mucous membrane – non encapsulated strains of *H.influenzae*, *H.parainfluenzae*
- **Invasive** – infections connected with bacteraemia – encapsulated strains of *H.influenzae* – in 98% is found type b (Hib)
  - meningitis, sepsis, prim. pneumonia, arthritis, cellulitis
  - age distribution from 6th mnths – 3rd year



# Diseases



# Laboratory diagnosis

- Sampling
  - throat swabs, sputum, pus, blood cultures, cerebrospinal fluid (CSF), aspirates from joints, middle ears or sinuses
- Microscopy
- Cultivation on enriched BA, chocolate agar, with *S.aureus*, with *growth factors*
- A positive oxidase test
- A positive catalase test
- Rapid latex agglutination test

## ATB susceptibility *H. influenzae*

- **ampicillin** (or amoxicillin), tetracyclines.
- **co-amoxiclav**, ciprofloxacin, azithromycin and **clarithromycin**
- **Ceftriaxone** (or a related cephalosporin such as cefotaxime) is the antibiotic of **first choice for the treatment of meningitis and acute epiglottitis**. It is bactericidal for *H. influenzae*, achieves good concentrations in the meninges and cerebral tissues, and is highly effective.

Prevention – vaccination against Hib

# Key points *Haemophilus*

- *H. influenzae* type b (Hib)
  - major human pathogen that causes invasive infections, including meningitis and epiglottitis
- Most strains of *Haemophilus influenzae* are noncapsulate but some strains possess a polysaccharide capsule (types a–f)
- Non-capsulate strains cause
  - non-invasive respiratory infections, including otitis media and acute exacerbations of chronic obstructive airway disease
- 15–20% of *H. influenzae* strains are ampicillin resistant ( $\beta$ -lactamase-mediated); ceftriaxone is the treatment of choice for invasive disease
- Conjugate Hib vaccine is administered as prevention

# Key points *Haemophilus*

- *H. ducreyi*
  - chancroid, sexually transmitted genital ulcers
- *H. parainfluenzae* and related microorganisms
  - occasionally implicated in human disease, infective endocarditis, but also dental infections, lung abscess and brain abscess

# Sources

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