Genus *Bordetella*, *Haemophilus*, *Neisseria*

Genus Bordetella

- Very small, gram-negative coccobacilli with capsules, non sporeforming

Significant species:

- *Bordetella pertussis*
- *Bordetella parapertussis*

  – causative agents of whooping cough in humans (pertussis, parapertussis)

- *Bordetella bronchiseptica* – pathogenic for animals

Bordetella pertussis

- not growing on basic culture media (toxic parts of agar)
  Cultivation on solid media: activated carbon, blood, albumin, starch, nicotinamide

- **Stainer-Scholte** enriched liquid medium - scientific purposes, improves the yield in isolation from clinical specimens
- **Bordet-Gengou agar** – classical solid culture medium (25% sheep blood, potato extract with glycerin) is prepared fresh (approximately 7 days)
- Cultivation time: 3-5 (7) days, very small, pearly gloss colonies, may have the narrow zone of hemolysis
- Colonies of *Bordetella* are identified by agglutination with specific antiserum

- Oxidase +
- Urease -

Bordetella parapertussis

- Growth on standard culture media (chocolate blood agar, blood agar, sometimes on McConkey) incubation of 1-3 days, larger colonies, significant hemolysis, colonies surrounded by brown pigment
- Oxidase –
- Urease +

Bordetella pertussis

Pathogenesis: name Pertussis (in Latin severe cough)

transmission: droplet infection (100 - 150 cm transmission of droplets when coughing)

Incubation time: 7 – 10 days, (ranging from 1 to 3 weeks), 22% of household contacts – extended incubation time up to 28 days

Untreated person is contagious: 6-8 weeks

Three stages: catarrhal, paroxysmal, convalescent

The disease is highly contagious - 80 - 100% of susceptible contacts of the patient become infected

- The protective level of antibodies against B. pertussis drops down to the cut off level within 3 – 5 years (even up to 12 years, depends on vaccine type
- Adult people have a mild form of pertussis in average 2.6 times per lifetime

Three stages: catarrhal, paroxysmal, convalescent

1) Catarrhal stage: like the common cold (runny nose, fever, fatigue, loss of appetite)

The determined diagnosis of pertussis does not mean that a patient is infectious, *Bordetella* can be cultured from the nasopharyngeal swab.

2) about 1-2 weeks - paroxysmal stage: severe spells of coughing, productive cough - abundant mucus, dyspnea, cyanosis, wheezing bout ends touch, sometimes vomiting.

Up to 40 to 50 seizures per day

dg. significant lymphocytosis

3) Convalescent stage - fewer cough, threatening secondary complications (lung infections, neurological complications ...)

B. pertussis is very sensitive to external environment

It is destroyed by:

sunlight

heat

Desiccation

common disinfectants

Regular cleaning, sufficient ventilation in the room

Epidemiological situation:

- Worldwide, emerging infection, recorded "return" of whooping cough
- according to epidemiologists only a low number of cases is reported (many cases are treated without accurate diagnosis
- There were even reports of deaths of unvaccinated infants

pathogenesis: *Bordetella* adheres to ciliated epithelium – multiplication

- local mucosal damage + production of toxins in the body (bronchitis, peribronchial inflammation to interstitial pneumonia) – inhabits exclusively columnar epithelium cilia in the airways
- systemic manifestations of the disease are caused by the toxin.
- The adhesion to a cylindrical epithelia – adhesins (filamentous hemagglutinin) and fimbriae --- *Bordetella* quickly adhere to columnar epithelium and block function of cilia

produced toxins

1) Pertussis toxin - regulates adenylate cyclase host cells = mucus production, inhibition of phagocytosis, amplifies some hormonal activities - hypoglykemia, increased sensitivity to histamine and enhances capillary permeability

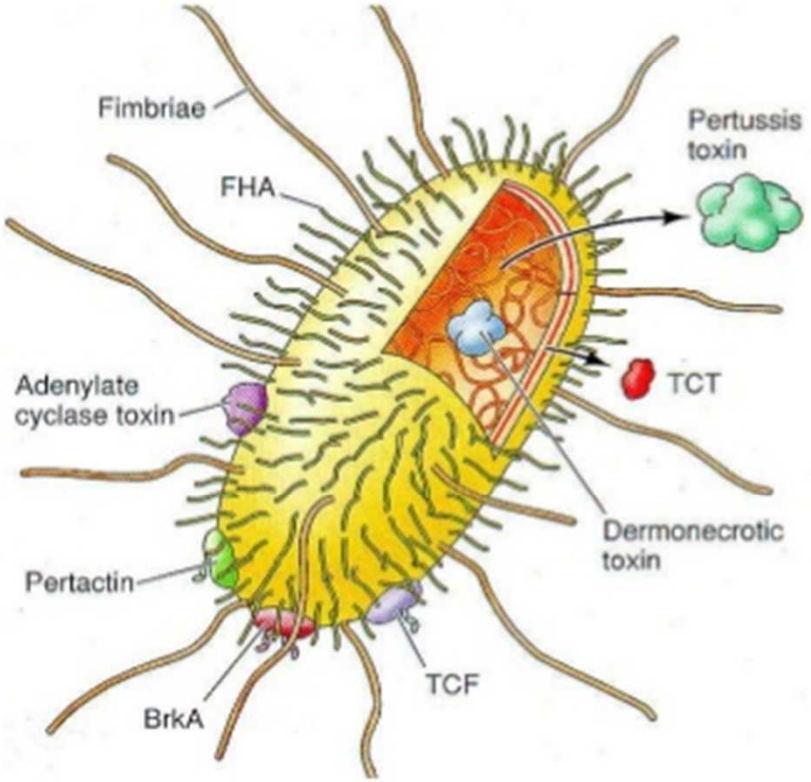
produced toxins

- Tracheal cytotoxin - toxic to ciliated epithelium
- Adenylate cyclase / hemolysin
- Lipopolysaccharide (LPS) - endotoxin - effect as LPS G- bacteria
- Lethal (dermonecrotic) toxin – responsible for local necrosis

Ed. The mutants are avirulent without adenylate cyclase

Bordetella pertussis

- Gram-negative bacterium
- Must attach to host cells to survive
- Virulence factors damage host tissue
- Contains LPS with unusual structure



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Therapy

- the catarrhal stage of disease can be positively affected by antibiotic therapy
- Azithromycin (macrolide) - the ATB of choice

Epidemiology

Disease - only in humans
Transfer - infectious aerosol (droplet infection)
worldwide over 60 million cases per year

- Infection in adults - decrease of antibody level after vaccination

they are a dangerous source of infection for children (pertussis in adults do not have typical symptoms)

- Vaccination - a part of a multivalent vaccine

Laboratory diagnosis

- **Direct diagnostic:**
- Microscopy and immunofluorescence tests are less sensitive
- Culture is important - nasopharyngeal swab
  - Bordet Gengou medium, temperature +35° - 37°C
  - suspected colonies - Gram staining method, agglutination with antiserum
- PCR method – rapid, sensitivity of 80% and more

- **Serology:** detection of antibodies (e.g. filamentous hemagglutinin, pertussis toxin), ELISA, agglutination
- 4-fold increase in titer

Genus *Haemophilus*
Family Pasteurellaceae

small gram negative rod (less than 1μm in diameter), non-motile, pleomorphic , no-sporulating

Nonpathogenic and pathogenic, occur on the mucous membranes of humans and animals

Since 1984 are included in the family*: Pasteurellaceae*

important members: *H. influenzae, H. aegyptius, H. ducreyi*

Less prominent members: *H. haemolyticus, H. parainfluenzae, H. parahaemolyticus* - potentionally pathogenic, can cause respiratory diseases, endocarditis, sepsis rarely

Culture conditions: higher tension of CO2 (5-10%), 37° C, the dependence on growth factors

Haemophilus is catalase positive, except *H.ducreyi* they are also oxidase positive

Virulence Factors of *Haemophilus influenzae*

- **Capsule**
  - There are 6 serotypes of the capsule (a-f).
  - **Type b capsules are the most important in pathogenesis**. Organisms that have capsule type b cause meningitis and other invasive diseases. This capsule is composed of a polymer of **polyribosyl ribitol phosphate (PRP)**, an oligosaccharide.
  - Antibody specific for the capsule is protective.
- IgA1 protease
- Endotoxin (LPS)
- Outer membrane proteins involved in iron acquisition
- Pili and other adhesins

Epidemiology

- *Haemophilus influenzae* is spread via respiratory droplets.
- *Haemophilus influenzae* type b (Hib) can cause invasive disease (i.e., it can get into the bloodstream and cause diseases like meningitis.)
- *Haemophilus influenzae* with capsule **type b causes meningitis, septicemia, epiglottitis, and pneumonia.**
- Non-typable *H. influenzae* (NtHi) strains cause pneumonia, otitis media and sinusitis.

- Disease is primarily seen in **children less than 5 years old who have not been immunized or older people with chronic bronchitis**

Dependence on growth factors

Haemophilus influenzae is a fastidious organism.

Genus *Haemophilus* depends on:

X-hemin

V-nikotinamidadenindinukleotide (NAD)

Haemophilus –growth factors must be added into the medium

Dependence can be demonstrated eg. using saturated discs and media without factors - grows around discs

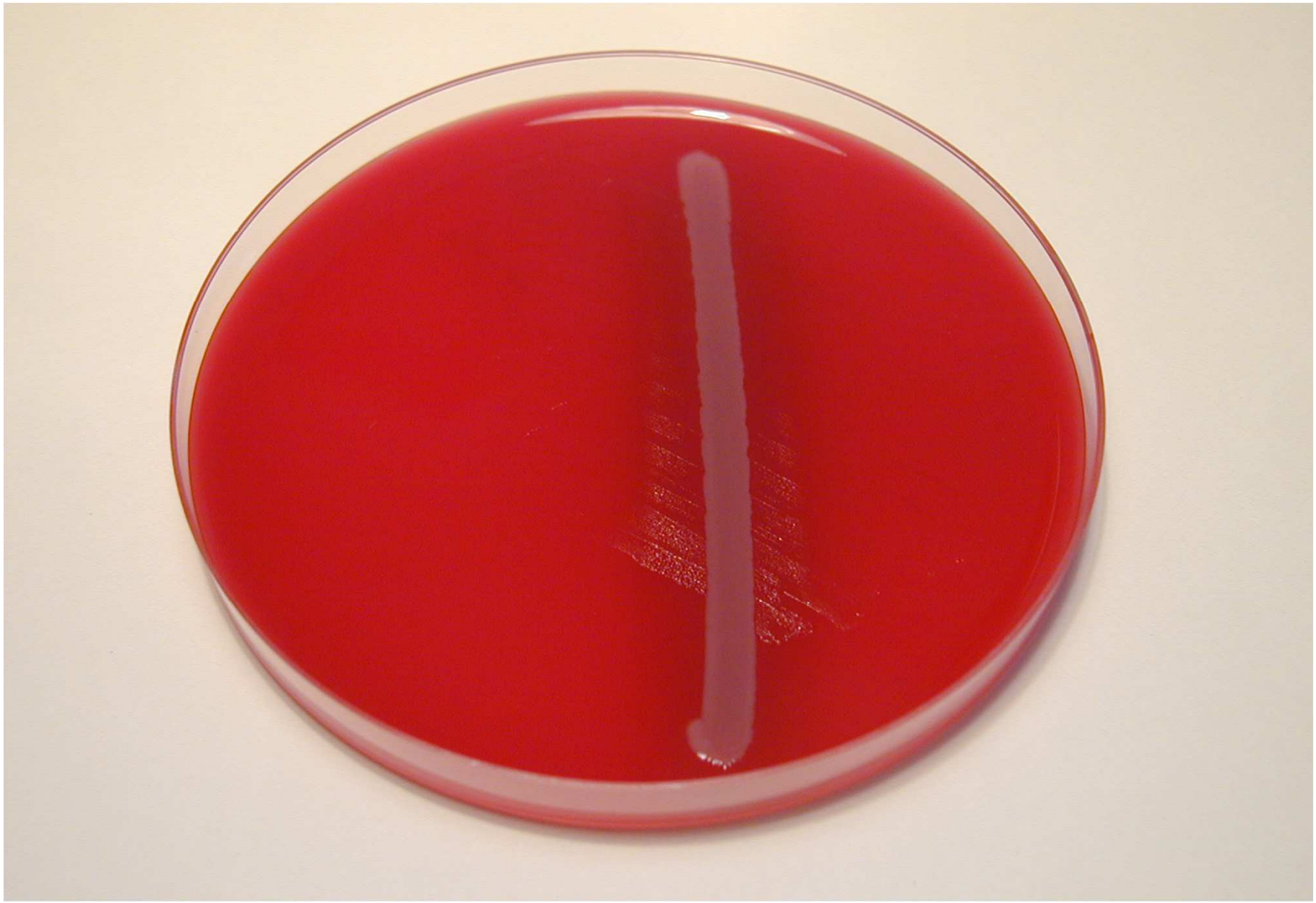
H. influenzae needs both factors (X and V)

H. parainfluenzae only factor V

- ***Haemophilus influenzae* can be cultured on a variety of media:**
  - chocolate agar
  - blood agar but with a *Staphylococcus aureus* cross-streak to release V Factor. Growth of the tiny *H. influenzae* colonies growing near the large *Staphylococcus* colonies is called satellitism.

Haemophilus on chocolate agar

Haemophilus – satelite phenomenon on blood agar



Haemophilus influenzae

pathogenesis: droplet infection, contact

development of the disease only in a part of infected - mucosa damaged primary viral infection, smoking, COPD

Main virulence factor - the capsule (6 serotypes, the highest virulence - type b)

Haemophilus lipopolysaccharide (LPS) – ciliostatic - damages the columnar epithelium, some strains produce protease - breaks IgA - a weakening of mucosal immunity

H. influenzae type b - colonizes URT

Clinical outcome: pharyngitis and sinusitis to severe pneumonia and epiglottitis (dangerous)

May penetrate through the epithelium into the blood vessels - bacteremia - meningitis, septic arthritis, pericarditis

acute epiglottitis: mostly 2-5 years, high temperature, acute swelling of the risk of mechanical airway closure (especially touch), severe sore throat

Haemophilus influenzae

Acute bacterial meningitis: type b, a serious disease of children - especially under 2 years of age, entry by penetration of epithelial cells from the nasopharynx into the blood or lymph and entry into the CNS.

Other *Haemophilus influenzae* serotypes are less pathogenic and are causative agents of local respiratory tract infections (pharyngitis, bronchitis ... ..) otitis, konjunctivitis, sinusitis…

Especially at: damage to mucosal viral infection, COPD (chronic obstructive pulmonary disease). . .

Characteristic are: cough may last for weeks

Haemophilus influenzae

laboratory diagnosis

Biological material (according to clinical manifestations)

nasopharyngeal swab, pus, punctate, blood, cerebrospinal fluid

Haemophilus are sensitive to external influences, require the use of transport media, biological material is stored at room temperature – sensitivity to cold (at 4 ° C rapidly killed)

Culture on chocolate agar or blood agar with a „streak" of *Staphylococcus aureus* laboratory strain – suspected colonies perform satelitism test

also porphyrin test , MALDI e.t.c.

Agglutination of the strain: bacteria grown from isolated colonies + antiserum

Therapy: Amoxicillin (ampicillin) - Some isolates of *Haemophilus influenzae* produce β-lactamases; cefotaxim/cetriaxon (for systemic infections)

Alternative: chloramphenicol, amoxicillin+clavulanic acid (β-lactamase positive strains), cotrimoxazole

Prevention

- hexavalent vaccine (Infanrix Hexa)

Composition conjugate vaccine:

- Diphtheriae anatoxinum
- Tetani anatoxinum
- Antigens of *Bordetella pertussis*
- Antigen of hepatitis B
- Virus poliomyelitis (inactivated) – 3 strains
- *Haemophilus influenzae* type „b“ polysaccharid (from capsule)

Genus *Neisseria*

Gram-negative, anaerobic and microaerophilic cocci

The genus includes two important human pathogens:

1) *Neisseria gonorrhoae* („gonococcus“)
2) *Neisseria meningitidis* („meningococcus“)
3) A group of non-pathogenic (rarely opportunistically pathogenic) neisseria, that occur in the oral cavity and nasopharynx "oral Neisseria„

Morphology: Gram-negative diplococci, clinging to each other flattened side - shaped "coffee bean"

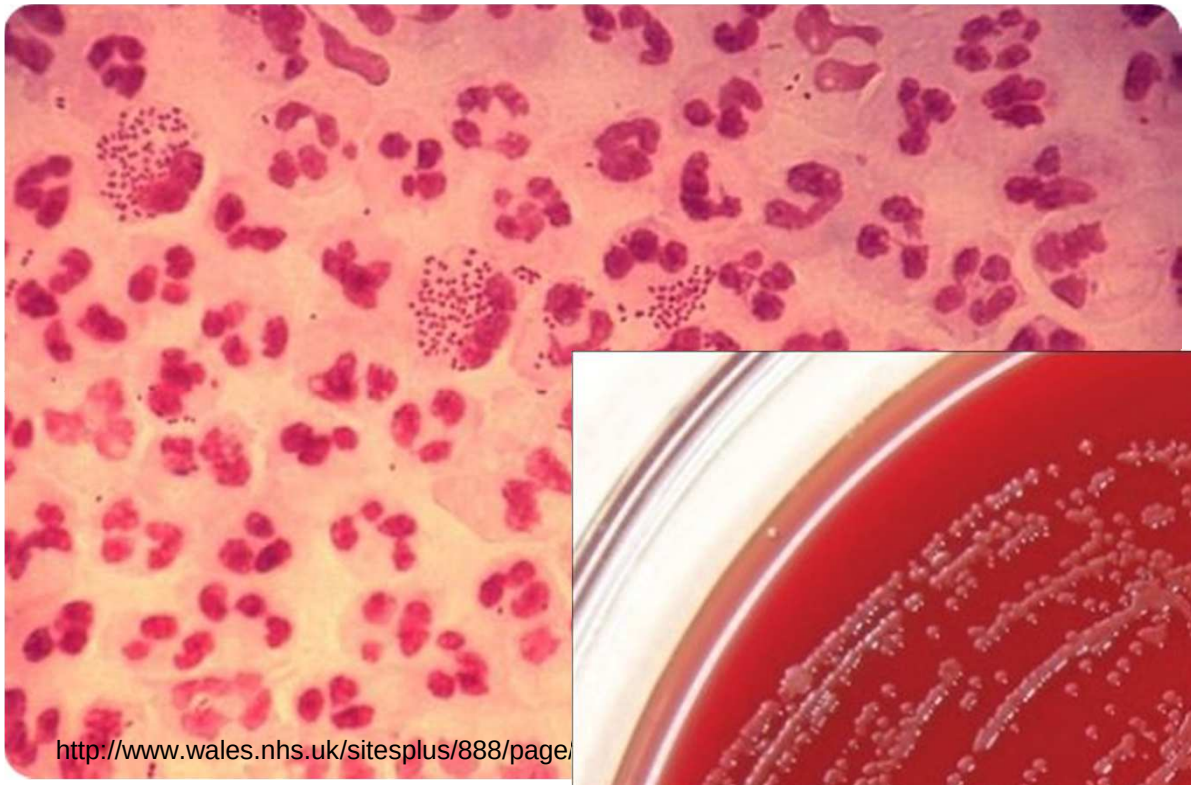
Biochemical properties:

oxidase positive
catalase positive

Usually utilize a few carbohydrates

Gonococcus only glucose

Meningococcus glucose and maltose



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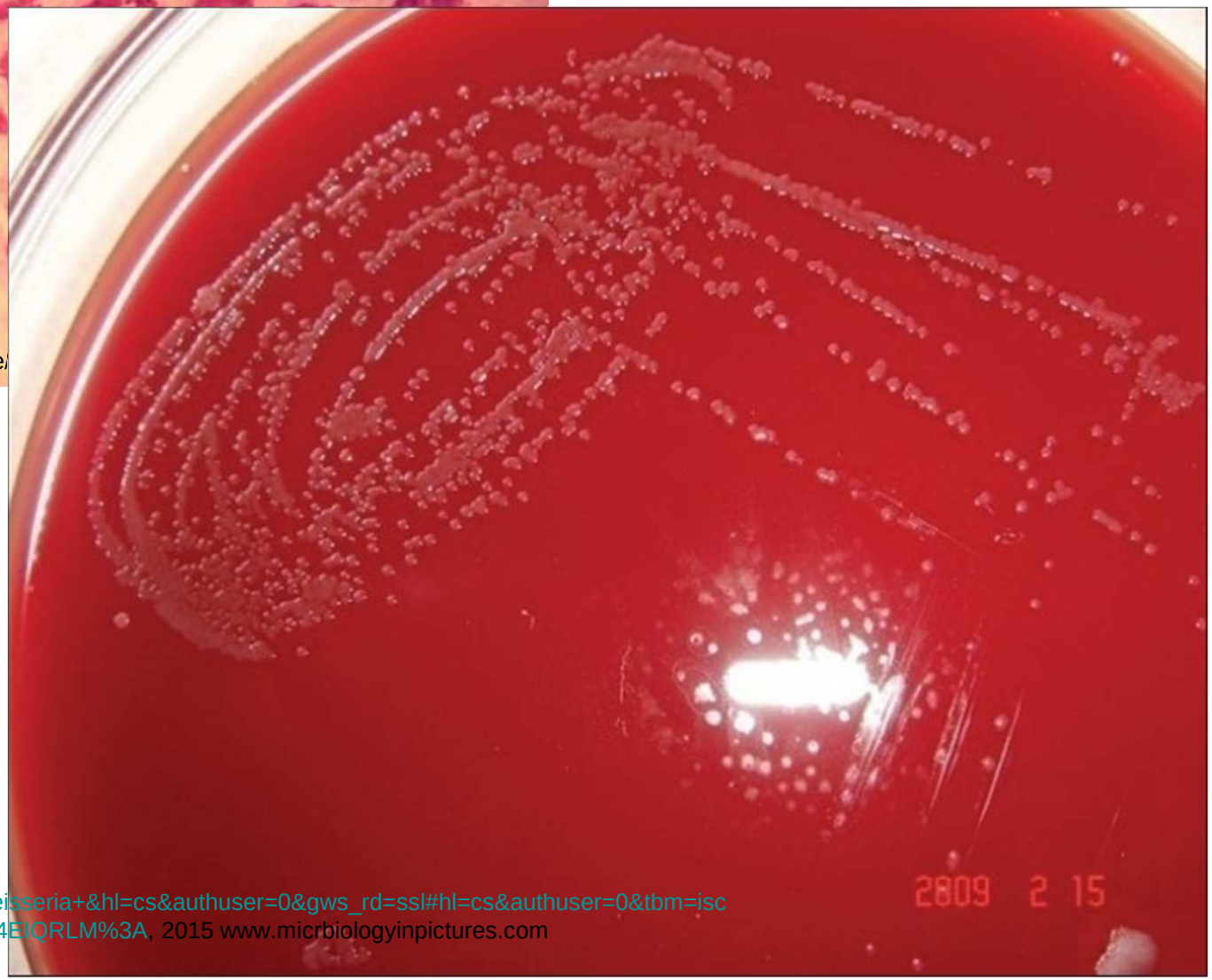

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medically important

Neisseria gonorrhoae

Obligate pathogens, the causative agent of gonorrhea, morphology have meningococcus and gonococcus same

In biological material - in the number of leukocytes often intracellularly - has the ability to resist after phagocytosis destruction

Gonococcus is sensitive to culture medium composition and to external influences

Cultivation: GO medium (enriched chocolate agar with antibiotics inhibiting the growth of other bacteria) - 24 - 48 hours rather, it requires a higher tension CO2, sensitive to desiccation

In vitro properties copied properties in vivo - transmission only by sexual contact

They are sensitive to weak disinfectants

At least biochemically active is gonococcus

Neisseria gonorrhoae

Pathogenicity factors:

Pili - fimbriae: very tight binding of bacteria cells in urethra, resistance also to phagocytosis and antibody activities – gonococci are able "gene conversion" on and switch off genes that encode growth fimbriae, against whom he organism produces antibodies and turn on the effect of other genes.

virulence factors – attaching ability on cells of the host organism, defense mechanisms against the immune system of the host - fimbriae, special proteins, transferrin and lactoferrin - cell wall lipopolysaccharides, opacity factor, porin, hypervirulent strains' pathogenicity islands "in the chromosome“

Infective dose: a woman of about 10^2 -10^7 CFU

a man of about 10^3 CFU

People with immune deficiencies are at risk of systemic diseases - bacteremia, fever, skin lesions, arthritis, endocarditis

Newborns - are infected during birth by vaginal delivery - purulent conjunctivitis (ophthalmia neonatorum) - prevention is: testing of pregnant women

The risk of transmission during one unprotected intercourse with an infected person is according to the WHO 50% of women and 20% men

Pathogenicity: only to humans, chimpanzee can be experimentally infected

illness: gonorrhoea

Classical venereal disease – non-invasive inflammation of urogenital mucosa

incubation: 2 – 7 days

clinical manifestations: especially purulent discharge from the urethra (men and women) = urethritis, cervicitis in women as well.

pharyngitis, tonsillitis – If gonorrhea is not in diagnostic considerations and is not a requirement, the laboratory has no reason to use GO medium (enriched) and gonococcus can not grow !!!.

Another form – homosexual men - requires taking swabs from the pharynx and rectum and culturing at GO medium…,

Swab from the rectum with ATB - suppression of contaminating flora from stool

Complications in women - infection may be spread per continuitatem to the pelvic organs - salpingitis, peritonitis ..... - Used the term DGI = disseminated gonococcal infection

Neisseria gonorrhoae

Laboratory diagnosis

Taking appropriate samples: from urethra, and / or cervix

Pharynx - suspected reservoir

Transport in the transport medium - very sensitive bacteria, reduce thermal shock

1) smear on the slide (then Gram stain)
2) Cultivation - GO agar (enriched medium), preheated to the room temperature or better 37°C - preventing thermal shock, small colonies in 48 hours, catalase + oxidase +, utilize glucose
3) PCR detection

Neisseria gonorrhoae

therapy:

Testing of susceptibility to antibiotics:

Geographically, there are different resistance profile

penicillin - not used (betalactamase production)

High prevalence of resistance to fluoroquinolones (not indicated for primary therapy)

Therapy - cephalosporins III. generation - ceftriaxon i.m.

Neisseria meningitidis

Meningococcus

- G negative diplococci, "coffee beans"

- It is less sensitive to the culture conditions than gonococcus, but more than an "oral Neisseria„

Parallel: - GO agar culture and blood agar (enriched with growth factors, Biochemically active low - only breaks down glucose and maltose oxidase +

Biological material for cultivation: cerebrospinal fluid, blood

Neisseria meningitidis

Pathogenicity: includes full scale - symptom less carrier of the pharynx (indicate 10% of the healthy population) to fulminant course of disease

- Non-invasive infections: mostly as pharyngitis
- Invasive infections: sepsis, meningitis - and combination of them

- The affected age group are mostly young people around 20 years of age,
- In the pathogenesis and virulence factors, host status - immunity, current physical condition (untrained young person for a greater physical effort - dance, sports performance)

  condition of the mucosa - smoking, dryness of mucous membranes

Into the bloodstream meningococcus probably from gets mucosa pharyngeal / laryngeal area

To the CNS presumably haematogenous route - is not excluded penetration through lamina cribrosa.

Neisseria meningitidis

Antigenic equipment: capsular polysaccharide antigens - meningococcus are distinguished by this antigenic structures to the serotype A, B, C, W, Y, Z.

Pathogenicity factors: IgA protease, porins, adhesiveness factors: fimbriae (significant) and opacity factors

Notable are antigens of capsule: Defense of organism is possible only in the presence of complement - for people with the disorder - greater risk

Capsule is compound from components similar to host polysaccharides - difficult defense, poorly immunogenic

Clinical symptoms

- The first are flu – like symptoms
- The temperature can go up rapidly up
- meningeal symptoms
- The typical rash (petechiae - and extensive bleeding into the skin)
- Fully developed sepsis - up to 20% of the infaust prognosis (disseminated intravascular coagulopathy, metabolic disorders).

Neisseria meningitidis

Laboratory diagnosis:

cerebrospinal fluid (CSF) and blood

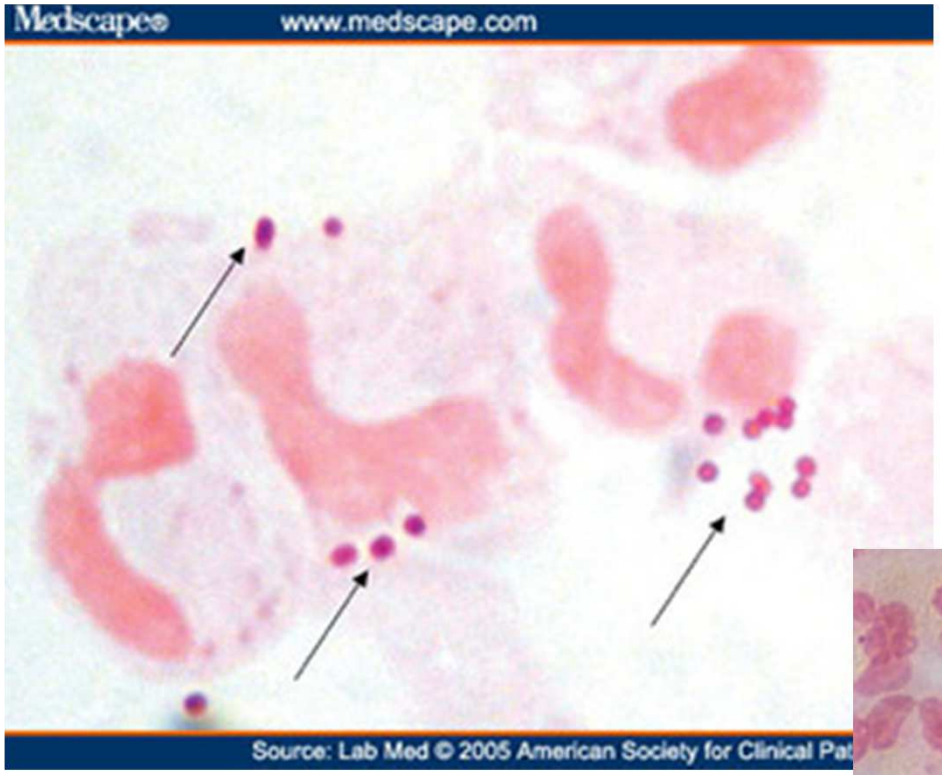
CSF: processed immediately

- Gram staining method - after centrifugation of sediment (quick result if positive)

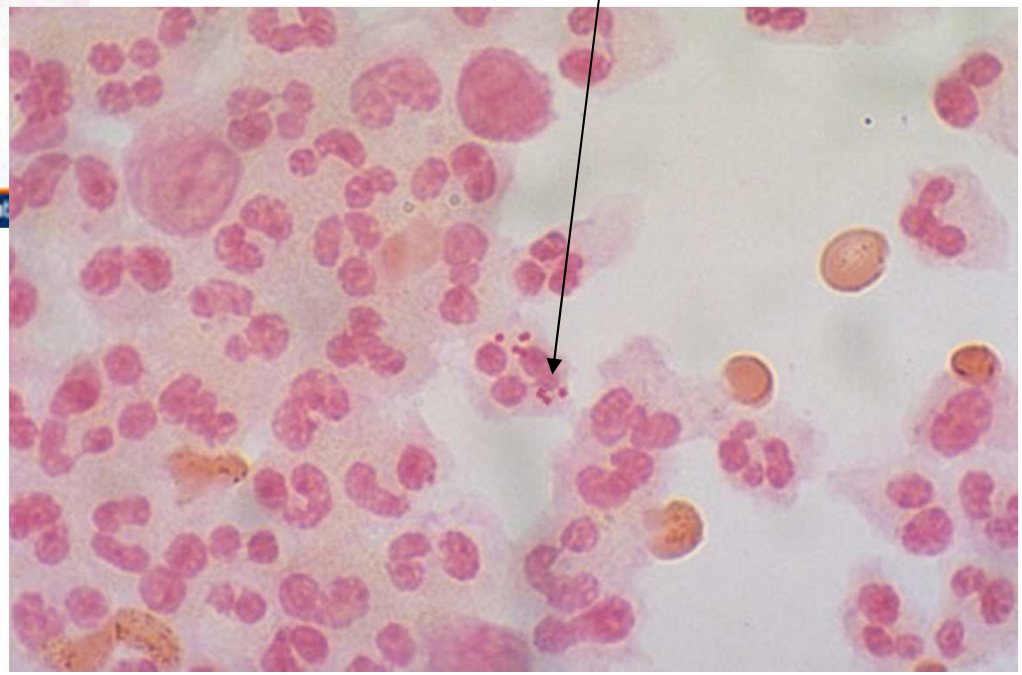
- Latex agglutination soluble antigen - supernatant / quick result (if positive)

- Cultivation of sediment - GO agar, blood agar, enrichment broth and re-inoculation again, isolation, identification

- PCR



Microscopically, a gram stain reveals gram negative diplococci within a neutrophil, typical for *Neisseria meningitidis*. Gram stain and culture can be performed on cerebrospinal fluid obtained via lumbar puncture.



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http://news.sciencemag.org/health/2012/05/death-california-researcher-spurs-investigation

Neisseria meningitidis

Antibiotic susceptibility testing

Therapy: Ceftriaxon, Cefotaxim, Benzylpenicillin

Prevention: conjugate polysaccharide vaccine (polysacharide from capsules)

Vaccination A, C, W, Y
newly B