Neisseria, Bordetella, Haemophilus, Legionella

GENERAL FEATURES

Gram-negative non-sporeforming bacteria. Some bacterial species (oropharyngeal Neisseria spp.) are part of normal flora. Some bacterial species may produce virulence factors and be pathogenic.

VIRULENCE FACTORS & PATHOGENESIS

Virulence factors are genetic, biochemical, or structural features that enable an organism to cause disease.
NEISSERIA. Hair-like pilli are essential for attachment to mucosal surfaces and confer resistance to phagocytosis. Over time the antigenicity of the pilli changes due to structural genes conversion. IgA protease cleaves IgA_1 inactivating it. Lipooligosaccharides (LOS, analog of LPS in other gramnegative species) is responsible for the endotoxic activity of *Neisseriae*. BORDETELLA. *B. pertussis* produces the filamentous hemagglutinin which mediates the bacteria's attachment to ciliated epithelial cells. Adenylate cyclase toxin decreases chemotaxis and phagocytosis. It increases cAMP levels which results in an increase of respiratory secretions and mucus production. This is characteristic of the paroxysmal stage of pertussis. Pertussis toxin is a bi-component toxin which causes lymphocytosis and inhibits the immune system. Dermonecrotic toxin causes localized tissue destruction. Tracheal cytotoxin causes ciliostasis and may even destroy the ciliated epithelial cells. HEMOPHILUS. The major invasive factor is it's capsule of which there are six serotypes (named a through f), IgA protease degrades secretory IgA to facilitate colonization of the upper respiratory tract mucosa.

INFECTIONS & EPIDEMIOLOGY

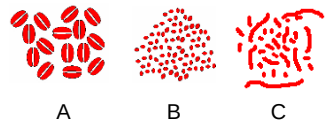
Neisseria meningitis is the cause of meningococcemia (septicaemia). This occurs when the bacteria crosses nasopharynx barrier and enters the bloodstream where it rapidly multiplies and causes petechial rash or haemorrhagic bullae. Within hours (up to 12) the infection could progress to fatal septicaemia.
Meningitis is the most common complication of meningococcemia. The disease occurs worldwide. Incubation period lasts 2-10 days. *Neisseria gonorrhoea* is one of the major causes of sexually transmitted disease (STD). It causes uretritis, endocervicitis, salpingitis (and infertility as a sequale) and opthalmia neonatorum. All of these infections are characterised by purulent discharge from the inflamed lesions. *Bordetella pertussis* causes whooping cough. Even if bordetella binds to upper respiratory tract large amounts of mucus are produced and inspired. This causes paroxysms, which may end in vomiting and cause cyanosis. There are three stages of the infection: catarrhal, paroxysmal and convalescent. *B. parapertussis* causes a similar disease but with milder clinical symptoms than can be seen in infections caused by *B. pertussis*. *Haemophilus influenzae* with capsule B (Hib) is the cause of serious invasive diseases especially in young children. Infections fall in two categories: 1. Otitis media, sinusitis, epiglottitis and bronchopneumonia, which result from the spread of the agent from the respiratory tract 2. Diseases following from invasion of the agent into bloodstream (meningitis, septic arthritis, cellulitis).

TREATMENT, PREVENTION & CONTROL

Neisseria meningitis infections. Treatment: Start of the therapy should not be delayed if high fever, headache and typical petechial rash occur in patients with meningococcemia. Drugs of choice are – high intavenous doses of penicillin G, ampicillin or ceftriaxon. Prevention: Children older than 2 years could be vaccinated with conjugative vaccine against serogroup specific polysaccharades but specific a vaccine for B serogroup is not available. *Neisseria gonorrhoea infections.* Treatment: Penicillin G is the drug of choice but 20% of the strains are penicillinase-producing (PPNG), hence, are resistant to penicillin and also other antibiotics (tetracycline, cefoxitin). The resistance is determined by gene for beta-lactamase of the TEM type. The infection could, thus, be treated by intramuscular ceftriaxone. Prevention: a vaccine does not exist because of pili structural genes conversion (see above in the text); general prevention for STD infections could be applied. *Bordetella pertussis (B. parapertussis) infections.* Treatment with a macrolide is effective in eradicating organisms and reducing length of infectious stage. Prevention: Acellular vaccines (inactivated pertussis toxin) in combined DPT vaccine. Haemophilus infections. Treatment of localized infections can be accomplished by ampicillin and an inhibitor of beta lactamase or co-trimoxazol. For invasive infections (e.g. meningitis) the drugs of choice are 3rd generationn cephalosporins (e.g. Ceftriaxon). Prevention: carbohydrate conjugated vaccine against invasive infection of Hib.

LABORATORY DIAGNOSIS

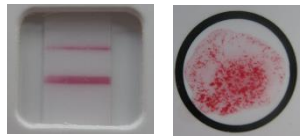
The decision to treat meningococemia, meningitis and pertussis must be based on clinical observation and must never be delayed!
a) Specimens: blood, cerebrospinal fluid, nasopharyngeal swabs and other clinical material depending upon localization infection.
b) Microscopy: bacteria with specific morphology (fig.1).



A B C

Fig.1. A. Diagram of gram-negative cofee-bean cells of neisseriae , B. small coccobacillary cells of bordetellae, C. polymorphic cells of hemophilae (C)

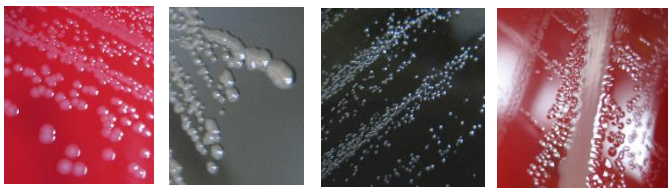
c) Direct detection of the infections with extremely rapid onset or great intensity has extraordinary significance (fig.2).



A B

Fig.2. Direct detection of Neisseria meningitis antigen in cerebrospinal fluid using enzymoimmunoanalysis (upper line – control reaction)(A) and agglutination test (B).

d) Culture: general enriched culture media (blood agar) and chocolate agar are used for cultivation of Neisseria and Haemophilus. Nutritionally fastidious bordatellae growth is enhanced and hemolysis potentiated by nutritionally rich Bordet Gengou agar on which the bacteria grow up to 7 days. Growth of competitive flora from the sample can be inhibited using selective agent (e.g. antibiotics) in the media (e.g. charcoal agar for bordetella cultivation)(fig.2).



A B C D

Fig. 2. A. Colonies of N. meningitis growing on blood agar, B., C. Bordetella pertussis and B. parapertussis, respectivelly, growing on nutritionally reach charcoal agar with cephalexin as a selective agent, D. Satellite growth of H. infuenzae - small transparent colonies growing around a vertical pigmented line of S. aureus

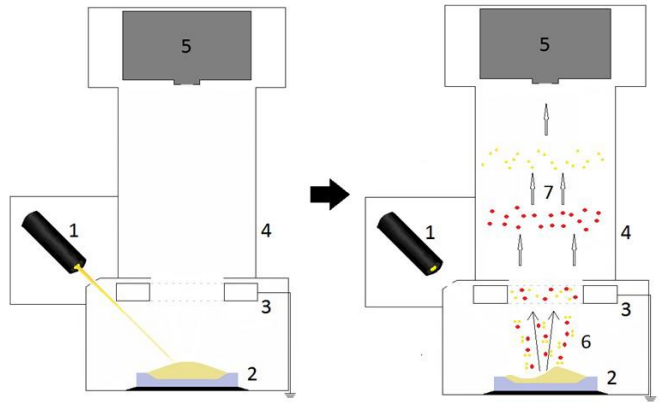
e) Phenotypical identification is focused on detection of specific biological properties of the agent (microscopy, bacterial colonies, enzymes detection).
Screening tests (preliminary identification): detection of some phenotypic properties are shown in fig.3.
Biochemical identification. Multiple enzyme tests are performed to identify the bacteria. Positive and negative reactions are noted. The metabolic profiles of known species are compared with the strain being analysed. Key dichotomous method and numerical identification are used.

Fig.3. Identification of clinical isolates of Neisseria using a diagnostic kit with various biochemichal substrates.

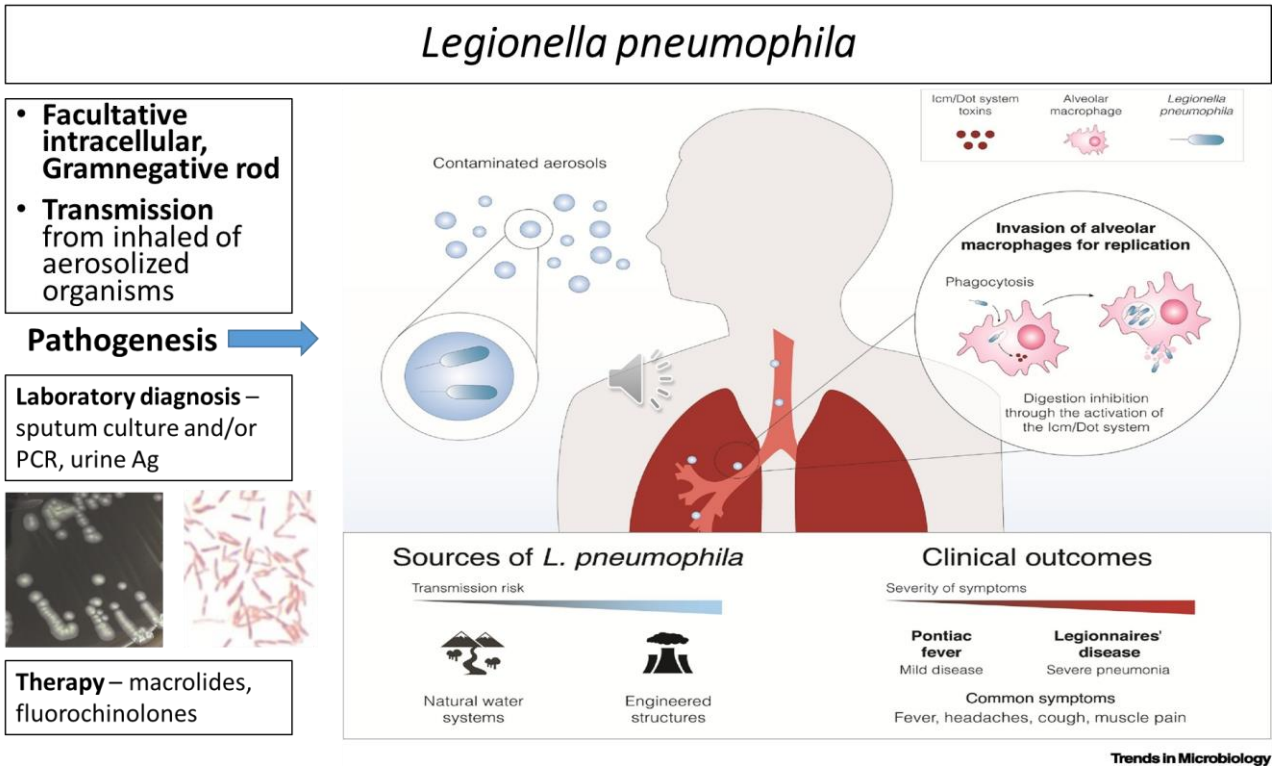
***Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry* - (MALDI – TOF MS):** is nowadays the most useful modern phenotypic method for microbial identification, which analyses molecular structure (mainly proteins) of an unknown microbial isolate, as the mass spectral pattern consisting of a number of structurally related mass spectral peaks, and comparing with a known patterns analyzing the isolate.

References: Murray – Medical Microbiology, 2005; Lippincotts Illustarted Reviews Microbiology 2007; Jawetz, Melnick and Adelsbergs Medical Microbiology, 2007

Fig. : **Sample preparation and principle of MALDI-TOF/MS analysis**
1. Sample of pure culture, 2. Pure culture inoculated on target plate and addition of UV-light absorbing matrix, which will absorb most of the energy upon irridiation with the nanosecond laser pulse, and thus prevent damage like fragmentation of the biomolecule, but will still allow the transformation of the sample molecules into ionized gas.
The mass spectrometer first ionizes, then mass separates and finally detects time of the ions flight, thus producing a mass spektrum and comparing with known mass spektra analyse the studied microorganism.
1. laser, 2. microbial culture on target plate, 3.strong electric field, 4. time of flight (TOF) tube, 5. detector of time of flight, 6. ionized molecules of sample (red) and matrix (yellow), 7. separation of accelerated particules.



f) Genotypic identification. There are several ways to identify a strain or species using genotype. For instance, comparing electrophoresed fragments allows visualisation of the restriction profile. Alternatively, homology of highly conserved regions, such as the 16S RNA gene, can assist identification of the species.
g) Susceptibility testing. Qualitative (disk diffusion method), quantitative methods or their combination (Etest) are used to test the susceptibility



PRACTICAL PART – NEISSERIA, BORDETELLA, HAEMOPHILUS

1. MICROSCOPY: prepare a gram-stained smear from purulent urethral discharge from a male with a gonococcal infections (or from a pure culture of *N. gonorrhoeae*). After drying, fixing and gram-staining draw the morphology of the bacteria you can see in the microscope.

2. MICROSCOPY AND PRELIMNARY IDENTIFiCATION: Perform selected phenotypical screening tests with a suspected culture of *N. meningitis* and/or *N. gonorrhoeae*. After drying, fixing and gram-staining of the cultures draw the colony's morphology you can see in microscope view. Does the morphology of the cells vary?

3. CHARACTERIZE CULTURES OF BORDETELLA PERTUSSIS (B. PARAPERTUSIS) AND HAEMOPHILUS INFLUENZAE. Specify which media are useful for their cultivation and specify which phenomena are seen.

4. PRELIMINARY IDENTIFICATION OF HAEMOPHILAE. Using disks containing hemin, V factor (nicotin amid dinucleotid or nicotin amid dinucleotid phospate) and X+V factors identify clinical strains of Haemophilus sp. Read results of disk diffusion test to analyze susceptibility of the strain to antibiotics.

LAB QUIZ

1. Specify virulence factors of neisseriae, bordetellae and haemophilae.
2. Specify infections caused by neisseriae, bordetellae and haemophilae. How should be diagnosed the infections and why?
3. Describe the principles of the treatment of the infections
4. Specify the direct detection methods used in diagnostics of the agents.
5. Specify the prevention and control of the infections.