Pyogenic cocci
(Neisseria)
Pyogenic cocci

- Spherical-shaped bacteria that are able to cause purulent inflammation of the mucous membranes of serous cavities (abdomen, pleura, pericardium) or in deep tissues (purulent infiltration, phlegmon).
  - *Staphylococcus* sp.
  - *Streptococcus* sp.
  - *Neisseria* sp.
Cocci

- A ‘coccus’ is a spherical bacteria
- *Staphylococcus* sp. tend to cluster in grape-like irregular bunches
- *Streptococcus* sp. tend to line up in chains
- *Neisseria* sp. tend to form a kidney-shaped diplococcus morphology
Overview of bacterial infections

- **Bacterial meningitis**
  - *Streptococcus pneumoniae*
  - *Neisseria meningitidis*
  - *Haemophilus influenzae*
  - *Streptococcus agalactiae*
  - *Listeria monocytogenes*

- **Otitis media**
  - *Streptococcus pneumoniae*

- **Pneumonia**
  - Community-acquired:
    - *Streptococcus pneumoniae*
    - *Haemophilus influenzae*
    - *Staphylococcus aureus*
  - Atypical:
    - *Mycoplasma pneumoniae*
    - *Chlamydia pneumoniae*
    - *Legionella pneumophila*
    - *Tuberculosis*
    - *Mycobacterium tuberculosis*

- **Eye infections**
  - *Staphylococcus aureus*
  - *Neisseria gonorrhoeae*
  - *Chlamydia trachomatis*

- **Sinusitis**
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*

- **Upper respiratory tract infection**
  - *Streptococcus pyogenes*
  - *Haemophilus influenzae*

- **Gastritis**
  - *Helicobacter pylori*

- **Food poisoning**
  - *Campylobacter jejuni*
  - *Salmonella*
  - *Shigella*
  - *Clostridium*

- **Skin infections**
  - *Staphylococcus aureus*
  - *Streptococcus pyogenes*
  - *Pseudomonas aeruginosa*

- **Sexually transmitted diseases**
  - *Chlamydia trachomatis*
  - *Neisseria gonorrhoeae*
  - *Treponema pallidum*
  - *Ureaplasma urealyticum*
  - *Haemophilus ducreyi*

- **Urinary tract infections**
  - *Escherichia coli*
  - *Other Enterobacteriaceae*

  - **Staphylococcus saprophyticus**
  - *Pseudomonas aeruginosa*
**Neisseria** genus

- *Neisseria* species are Gram-negative **human-specific** diplococci

- The genus *Neisseria* consists of commensal and pathogenic species that colonize the mucosal epithelia

- *Neisseria* species are naturally competent for DNA uptake
Neisseria infections

- The *Neisseria* sp. *N. meningitidis* and *N. gonorrhoeae* cause a variety of diseases from meningitis and septicemia / bacteremia (*N. meningitidis*) to urogenital tract and skin and joint infections (*N. gonorrhoeae*).
**Neisseria selective agar**

- Thayer-Martin agar
  - Mueller-Hinton agar with 5% chocolate sheep blood and antibiotics (VCN).
  - Inhibits the growth of most other microorganisms

- Selective antibiotics
  - Vancomycin => kills most Gram-positive organisms
  - Colistin => kills most Gram-negative organisms
  - Nystatin => kills most fungi
  - Trimethoprim => inhibits Gram-negative organisms, especially swarming Proteus
Neisseria meningitidis

- Strictly human
- Colonizes nasopharynx
- Causes meningitis and septicaemia
Acute bacterial meningitis

- The causative agent
  - *Neisseria meningitidis*,
  - *Streptococcus pneumonia*, *Haemophilus influenzae* type b

- Sign and symptoms
  - Fever, stiff neck, severe headache, vomiting and nausea, sensitivity to light

- Transmission
  - Respiratory droplets from prolonged contact

- Treatment
  - Antibiotics

- Prevention and control
  - Vaccination
Meningococcal disease

- Generally starts as local infection and then develops into a blood infection that finally invades the meninges
- Non-specific early symptoms
- Rapid progression to full disease (hours)
- Mortality rate up to 15%
- Sequelae: amputations, deafness, brain damage


CSF containing *N. meningitidis* and PMNs
Meningitis in infants

Meningitis Baby Watch

- Tense or bulging soft spot
- High temperature
- Very sleepy/staring expression/too sleepy to wake up
- Breathing fast/difficulty breathing
- Extreme shivering
- ‘Pin prick’ rash/marks or purple bruises anywhere on the body
- Sometimes diarrhoea
- ‘Pin prick’ rash/marks or purple bruises anywhere on the body
- Cold hands and feet

Is your baby getting worse fast?
Babies can get ill very quickly, so check often.

- Vomiting/refusing to feed
- Irritable when picked up, with a high pitched or moaning cry
- Blotchy skin, getting paler or turning blue
- A stiff body with jerky movements, or else floppy and lifeless

Sometimes diarrhoea

Cold hands and feet

Sometimes diarrhoea
Meningococcal disease is a global problem

USA: 1000-3000 cases per year*

Africa: 3000-10000 deaths annually*

Western Europe: ~8000 cases annually*

Annually +/- 500 thousand cases worldwide

Annually +/- 160 deaths in China

Capsular polysaccharide vaccines

- *N. meningitidis* is classified into 12 serogroups based on differences in the capsule structure

- Serogroups A, B, C, W-135, Y and X are most commonly associated with disease (>99%)

- *Neisseria meningitidis* vaccines contains capsular polysaccharide of four important serogroups (A, C, W-135 and Y) conjugated to inactivated toxin (toxoid)
  - Part of childhood vaccination schedules
  - During an outbreak or high risk groups (when military recruits enter boot camp, or for travellers to areas where meningitis is hyper endemic)
Non-immunogenic polysaccharide

- *Neisseria meningitidis* vaccine
  - In Western countries serogroup B has become most prevalent and it has replaced serogroup C
  - Serogroup B polysaccharide is identical to sugars expressed by our own cells and is therefore not immunogenic

Annual cases of laboratory confirmed meningococcal disease England & Wales 1992 to 2010 (up to 7th August 2010)
Serogroup B vaccine

- *Neisseria meningitidis* serogroup B vaccine
  - Contains a mix of several proteins (fHbp, NadA and NHBA) and a detoxified outer membrane vesicle (OMV) containing PorA.
  - The first vaccine developed by reverse vaccinology, which is based on screening immunogenicity of all putative surface proteins identified in the whole genome sequence.
  - Major drawback of this vaccine is the high sequence variability of the antigen targets in the bacterial population, which might limit its coverage.
Neisseria gonorrhoeae

- Strictly human
- Colonizes urogenital tract
- Causes the sexually transmitted disease gonorrhoea
Gonorrhoea

- Inflammation mucosal surface of the urethra, cervix, rectum, and pharynx
- Urethral infections: dysuria and purulent discharge
- Cervix, rectum and pharynx infections: generally asymptomatic
- When untreated: pelvic inflammation, arthritis, dermatitis, and endocarditis, ectopic pregnancy, sterility
- Development of antibiotic resistance
Development of antibiotic resistance

- *N. gonorrhoeae* has become a **superbug**
Spread of antibiotic resistant strains

The World Health Organization recommends that once a level of 5% resistance to an antibiotic is recognized, then that antibiotic should be removed from recommended treatment schedules for gonorrhoea.

Source: WHO
Global impact of Gonorrhoea

DALY rates from **Gonorrhea** by country (per 100,000 inhabitants) in 2004

Disability-Adjusted Life Year

Annually +/- 88-106 million cases worldwide
Annually +/- 3.8 million cases in China

Source: WHO

Reported cases of notifiable infectious diseases in 2007 in USA
Research intermezzo

- *N. gonorrhoeae* antimicrobial resistance in Hangzhou
**Antimicrobial susceptibility in Hangzhou**

- Analysis of the first 176 *N. gonorrhoeae* isolates

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Breakpoints (mg/L)</th>
<th>% of isolates (n=176) showing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Susceptibility</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>S≤0.125, R&gt;0.125</td>
<td>99%</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>S≤64, R&gt;64</td>
<td>100%</td>
</tr>
<tr>
<td>Cefixime</td>
<td>S≤0.125, R&gt;0.125</td>
<td>97%</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>S≤0.25, R&gt;0.5</td>
<td>82%</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>S≤0.06, R&gt;1</td>
<td>0%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>S≤0.5, R&gt;1</td>
<td>23%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S≤0.03, R&gt;0.06</td>
<td>0%</td>
</tr>
</tbody>
</table>
High-level azithromycin resistance

- High-level azithromycin resistance is defined by MIC $\geq 256$ mg/L
- High-level azithromycin resistant isolates have been isolated incidentally in several countries around the globe
- High-level azithromycin resistance poses a major threat for dual-antimicrobial therapies of ceftriaxone + azithromycin

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Breakpoints (mg/L)</th>
<th>No. (%) of isolates showing (n=176)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>$S \leq 0.25, R &gt; 0.5, HLR &gt; 256$</td>
<td>Susceptibility</td>
<td>Intermediate susceptibility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>82%</td>
<td>2%</td>
</tr>
</tbody>
</table>
NG-MAST molecular epidemiology typing

- NG-MAST assigns sequence types (ST’s) based on the combination of the highly variable genes *porB* and *tbpB*

- The 176 isolates belonged to 120 different ST’s

- The 23 high-level azithromycin-resistant isolates belonged to 8 different ST’s
High-level azitromycin resistant isolates

- ΔA in 13bp IR of \textit{mtrR} promoter
- A2059G mutation 23S rRNA
- SNP in MacAB efflux pump promoter
- Presence of \textit{erm} (rRNA methylases)
- Presence of \textit{mef}-encoded efflux pump
Conclusions

- High prevalence of high-level azithromycin-resistant *N. gonorrhoeae* isolates in Hangzhou, China

- These findings could have major implications for future antimicrobial treatment strategies in China

- A more thorough nationwide overview on azithromycin susceptibility is necessary before it can be included as a first-line therapy in combination with ceftriaxone
**Neisseria** virulence factors

- *Neisseria* are human-specific bacteria
- Adapted to survival in the human body
- Many specific virulence factors for interactions with human counterparts
Capsule

- Capsule contributes to virulence of *Neisseria meningitidis*
  - Protects against complement deposition and complement mediated killing
  - Protects against recognition of surface antigens by antibodies
  - Protects against phagocytosis
  - Protects against antimicrobial compounds secreted by host cells
Type IV pili

- Mediate attachment to various cells and tissues
- Involved in micro-colony formation
- Twitching motility
- Natural competence (DNA uptake)
- Extensive phase (on/off) and antigenic variation
Opacity proteins (Opa)

- Promote attachment and invasion of human cells
- Binds with human CEACAM
- Bacterial aggregation
- Extensive phase and antigenic variation
  - *N. gonorrhoeae* contains 11 different Opa loci
  - *N. meningitidis* contains 4 different Opa loci
IgA protease (IgAP)

- Secreted protein expressed by *N. gonorrhoeae* and *N. meningitidis*
- IgAP cleaves the proline-rich (Pro-Pro-Y-Pro) hinge region of the heavy chain of IgA antibodies
- IgA is the most abundant antibody produced in association with mucosal membranes

(1) Stimulation of epithelial Toll-like receptors (TLRs) with microbial-associated molecular patterns activates MyD88-dependent signaling pathways that trigger upregulation of PIGR gene transcription. Activation of TLRs may also stimulate polymeric immunoglobulin receptor (pIgR) transcytosis.
(2) Newly synthesized pIgR molecules are sorted to the basolateral surface of epithelial cells.
(3) Dimeric IgA secreted by lamina propria plasma cells binds to pIgR on the epithelial membrane and stimulates transcytosis.
(4) IgA-bound and unoccupied pIgR are transcytosed through epithelial cells.
(5) Proteolytic cleavage of pIgR at the apical surface releases SIgA and free SC.
(6) Binding of SIgA and SC to luminal bacteria promotes association with the mucin layer and biofilm formation, and prevents direct access of bacteria to the epithelial surface.
Hemoglobin/Haptoglobin-Lactoferrin-Transferrin binding proteins

- *N. gonorrhoeae* and *N. meningitidis* contain human-specific Hemoglobin/ Haptoglobin, Lactoferrin and Transferrin binding proteins to sequester iron from the human host.

- Iron is an essential building block and co-factor for enzymatic activities.

- Limitation of free iron is a major strategy to inhibit bacterial growth.
Lipooligosachharide (LOS)

- Endotoxin that is highly stimulatory to the human immune system
- Protects against antimicrobial peptides
- Important for phagocytosis by neutrophils
- LOS sialylation (by enzyme Lst) prevents complement deposition and phagocytosis
- LOS modification by phosphoethanolamine (by enzyme LptA) provides resistance to antimicrobial peptides and complement
- Glycoform variability limits adaptive immunity
Factor H binding protein (fHbp)

- Surface lipoprotein ~29kDa
- Binds human complement factor H
  - Repressor of the Complement Alternative Pathway
Complement essential for host resistance

Classical Pathway
- C1
- C1qNH
- C4bp
- C3
- Properdin
- fH
- fI

Lectin Pathway
- MBL

C3 convertase C4b2a

C5 convertase C3bB / C4b2a3b

Alternative Pathway
- Amplification loop

C5b / C8 / C9

Complement mediated lysis
- Cell lysis
- MAC
- Polymerisation

Associated with increased susceptibility

Activation
Inhibition
Complement Factor H

- Factor H binding is a common strategy for microorganisms to escape the immune system.
Porin

- Outer membrane transmembrane barrel
- Nutrient acquisition
- Binds complement factor H, C3b, C4b and C4bp
  - Represses complement mediated killing
- Involved in cell invasion and intracellular survival
- Modulates ROS production and apoptosis
Complement interactions

(1) Lectin pathway

Nm PorB

(2) Classical pathway
Ag-Ab complex

C1 C4 C2

C3 convertase
C4b2a

C3

C3b

Properdin

(3) Alternative pathway
Cell of microorganism + Factor B, D and P(properdin)

C3 convertase
C3bBb

C5 convertase
C3bBbC3b/C4b2a3b

C5a

C5

C5b

+C6,7,8,9

C5b

C6

C7

C8

+C9

MAC → Cell lysis

Inhibition

Amplification
Oxidative stress protection

- KatA => catalase that detoxifies ROS & H$_2$O$_2$
- MntABC => takes up manganese, which quenches ROS
- Sod => converts superoxide
- Glt => l-glutamate transporter, intracellularly converted into glutathione, which maintain redox balance during ROS exposure
- RecA, MutY, Uvr => DNA repair after oxidative DNA damage
Other *Neisseria* virulence genes

- MtrCDE => exporter of antimicrobial peptides, bile, etc.
- FarAB => exporter of fatty acids
- PacA => modifies peptidoglycan to protect against lysozyme
Next lecture

- Enterobacteriaceae & Vibrios