Objectives

• Pathogenesis
• Epidemiology
• Diagnostics
• Antimicrobial susceptibility testing
• Resistance
• Surveillance
• Typing
**Neisseria species**

- 2 species primarily pathogenic to humans, *N gonorrhoeae* and *N meningitidis*
- >30 non-pathogenic species (*N lactamica*, *N sicca* etc). Usually commensals of upper respiratory tract

**Neisseria gonorrhoeae**

- Infects columnar epithelium
- Gram-negative, arranged in pairs (diplococci) with adjacent sides concave ‘kidney bean’
- Nonmotile, non-spore forming
- Fastidious, requires complex nutritionally enriched culture medium for growth and 5% CO₂
Gonococcal Pathogenesis

Surface structures mediating adherence to epithelium

- **Pili** - Attachment
- **Opa** (opacity-associated proteins) - Attachment / Invasion
- **Porins** - Internalisation / Acquisition of nutrients
- **LOS** (lipooligosaccharide) - Toxic to epithelial cells

- Attachment, Internalisation, Acquisition of Nutrients, Evading the immune response
- Immunity is not protective against multiple infections
- Phase and antigenic variation of surface components
Gonorrhoea prevalence in the UK

Natsal3: Population-based prevalence estimates, 16-44 yrs

Sonnenberg et al, Lancet 2013;382: 1795-806

Gonorrhoea in England

- 78m cases worldwide (2012)
- 40,000 diagnoses in England per year
- Key groups affected:
  - Black Caribbean populations
  - MSM
- Highly geographically concentrated
- Strongly associated with deprivation
- Perpetuation in population associated with higher rates of partner change – complex sexual networks, partner concurrency
Rates of gonorrhoea diagnoses by gender and age: England, 2017

Repeat infection with gonorrhoea: England, 2012-2017
Diagnostics

- Presumptive diagnosis by microscopy
- Confirmation by isolation of *Neisseria gonorrhoeae*
- Identification methods:
  - Biochemical
  - Immunological
  - Molecular
  - Protein methods
- Susceptibility testing to direct patient management / surveillance purposes
- Molecular diagnostics

Presumptive diagnosis: Microscopy

- Gram stained smear allows direct visualisation of GNDC in PMNLs
- Sensitivity >95% - urethral smears from symptomatic men
- Sensitivity 20-40% - asymptomatic men, women and with rectal smears: lower numbers of GNDC
- Specificity high (97%) with experienced personnel. False positives? Look at all tests in combination.
- NOT recommended for pharyngeal infections
Culture

- Isolation of *Neisseria gonorrhoeae* by culture
  - Diagnostic gold standard
  - Sensitivity & specificity can approach 100% in symptomatic men with urethral discharge
  - Lower sensitivity in asymptomatic men, women and extra-genital specimens
  - Provides viable organism for susceptibility testing
Culture

- Fastidious and fragile
- Very susceptible to environmental conditions (temp, oxidation, drying, toxic substances)
- Requires live organisms; influenced by:
  - Technique and swabs used for specimen collection
  - Conditions and duration of transport
  - Quality of culture medium; maintain GC but prevent overgrowth of hardier commensals
  - Incubation conditions

Direct culture

- Near-patient inoculation onto selective culture media plate, incubated with enhanced CO₂ (candle jar or incubator) in clinic, transported to lab
- False negatives:
  - Poor specimen collection
  - Delayed incubation
  - Poor quality control of plates
Non-nutritive transport

- Non-nutritive transport medium +/- charcoal (Stuart or Amies)
- Isolation rate at room temp >90% at 6 hours, poor after 24-48 hours
- Stored in fridge prior to transport to retard growth, preventing loss of viability

Culture medium transport systems

- Nutritive (growth) transport medium (Gono-Pak, InTray GC system)
- Maintains growth conditions and CO₂ during transport and incubation
- Pre-incubated at 36⁰C prior to transport
- Better for longer transportation times (but <2 days)
Selective agar

Thayer-Martin or New York City agar

Base medium (chocolate) supplemented with growth factors, plus antibiotics ‘VCAT’

Inhibit Gram positive organisms

- Vancomycin, 3 ug/ml or Lincomycin, 0.5 ug/ml

Inhibit other Gram negative organisms

- Colistin, 100 units/ml; Trimethoprim, 5ug/ml

Inhibit yeasts (eg Candida spp)

- Nystatin, 12.5 ug/ml or Amphotericin, 1ug/ml

Incubation

- Incubated immediately 35-37°C, humid atmosphere with 5% CO₂
- After 24-48 hours, typical colonies 0.5-1mm, grey-white, vary transparent to opaque, convex to flat
Identification of GC

Presumptive identification

- Gram negative cocci
- Oxidase positive
  - Paper strip impregnated with redox indicator
  - Detects Cytochrome C Oxidase
  - Dark blue/purple oxidized: Positive

Confirmation

- Biochemical
- Immunological
- Protein methods
Immunological tests

Phadebact Monoclonal GC Test

- Antibodies to gonococcal porin PorB
- Agglutination test
- Positive Result for GC
- Negative result for all other Neisseria and other bacteria
- False negatives can also occur

Matrix Assisted Laser Desorption Ionisation Time of Flight (MALDI-ToF)

- Mass spectrometry detects the mass-to-charge ratio of proteins to produce a unique mass spectral fingerprint
- Can identify bacteria direct from the agar plate
- Equipment costs high - cost of individual tests is low
- Rapid and easy to perform
Susceptibility testing

Minimum inhibitory concentration (MIC): lowest concentration of an antimicrobial drug that will inhibit growth of an organism in vitro.

The lower the better!

Breakpoints (set by organisations e.g. EUCAST)

- Categorise strains into sensitive / intermediate / resistant
- Category relates to chance of therapeutic failure (usually!)
- Depends on site of infection, host and antibiotic (PK/PD)
- Need pure, fresh culture from non-selective media
- Medium important; GC agar base with supplements
Agar dilution

“Gold standard”
- Quantitative (MIC): lowest concentration that inhibits growth
- Antibiotics in serial dilutions incorporated into GC agar
- Laborious, not suitable for routine work
- Good for surveillance

Gradient strip ‘Etest’

- Correlates closely with agar dilution
- Quantitative (MIC)
- Strips with antibiotic concentration gradient and calibrated MIC scale
Disc diffusion

- Qualitative; categories, no MIC
- Cheap
- Discs impregnated with known concentration of antibiotic
- ?Accuracy

Beta-lactamase detection

- Plasmid-mediated resistance to penicillin
- Nitrocefin disc: streak colonies onto disc, positive turns red (beta lactam ring hydrolysed)
Preservation of isolates

- Subculture every 48 hours on GC agar
- Chocolate agar slopes (<5 days transportation)
- Long-term storage cryobeads -70°C

Molecular diagnostics

- Nucleic acid amplification tests (NAATs); molecular detection of specific nucleic acid (DNA/RNA) sequences of *N gonorrhoeae*
- Variety of methods to produce multiple copies easily detected e.g. PCR, TMA, SDA
- Highly sensitive and specific; more true positives but a chance of some false positives
- CT/GC result from same test
- More sensitive than culture at extra-genital sites; rectum and pharynx
- Faster turn-around times, high throughput
- Uses non-invasively taken specimens, urines, self taken vaginal swabs
Molecular diagnostics: issues

- Specificity, particularly extra-genital sites with commensal Neisseria
- Should GC NAAT be used in low prevalence settings; PPV <90%?
- Is repeating or confirmation necessary?
- Provision of viable bacteria for AMR

Testing low prevalence populations

Sensitivity
Number of true positives identified

Specificity
Number of true negatives identified
Effect of prevalence on Positive Predictive Value

- **Screening**
  - Prevalence 1%
  - Sensitivity 99.5%
  - Specificity 99.5%
  - PPV 67%

- **Confirmation/Supplementary**
  - Prevalence 67%
  - Sensitivity 99.5%
  - Specificity 99.5%
  - PPV 99.8%
Confirmatory testing

Use of another test with different gene target

• Only if both targets are present: true positive result
• In most settings, PPV >90% only achieved by confirmatory testing

Repeat testing of same sample only checks reproducibility

Management of discrepant results?

Extra-genital testing

• No NAAT is licensed for extra genital sites but good validation data exists particularly for rectal samples
• Some older NAATs had given poor specificity due to cross-reaction with genes from commensal Neisseria; new generation of NAATs have improved specificity
• All positives must be confirmed by a supplementary test with a different target.
• NAATs have a superior sensitivity to culture in extra-genital samples.
Summary: Molecular testing

- The positive predictive value should be >90% and may require a confirmatory test in low prevalence populations
- NAATs have a superior sensitivity to culture for detection of gonorrhoea in extra-genital specimens
- All GC positive NAATs from extra-genital specimens should have a confirmatory test

What can new technologies offer?

- Nearer the patient testing
- Test and treat in one visit
- Decrease healthcare costs
- AMR molecular detection
Resistance

WHO Global Report on Antimicrobial Resistance:

*Neisseria gonorrhoeae* among 9 bacteria of international concern

What happened?
Microbiology of N. gonorrhoeae

What influences resistance?

Misuse of antimicrobial agents

- Treatment with sub inhibitory dosage
  - Inadequate dosage
  - OTC use
  - Incomplete course of therapy (including sex before cleared infection)

Correct / continual use

- Long term use as treatment of choice

Selection of mutants

Selective pressure

Antibiotic resistance in *N. gonorrhoeae*

- Versatile organism
- Highly competent for genetic exchange
  - Other *Neisseria* (e.g. in throat)
  - Other bacteria
- Mixed infection is thought to occur frequently.
- Adept at developing mechanisms of resistance to therapeutic agents

**Acquisition**

*Plasmids*
- Penicillin (PPNG): *tem*-1 (Haemophilus)
- Tetracycline (TRNG): *tetM* (Streptococci)

*Chromosomal*
- Penicillin/Cephalosporin (Commensal Neisseria)

**Selection**

*High-level, single step*
- Spectinomycin
- Azithromycin

*Additive, multiple steps (mutations in multiple genes)*
- Penicillin
- Ciprofloxacin
Mechanisms of antibiotic resistance

- **Modification of target**
- **Impermeable to antibiotic**
- **Efflux of antibiotic**
- **Enzymatic modification/ degradation of antibiotic**

**Azithromycin - mechanisms of resistance**

- **Modification of ribosomal target**
  - Mutations in 23S rRNA alleles
    - High-level >256 mg/L from mutations in at least 3 / 4 alleles
- **Efflux of antibiotic – up regulation of efflux pump – mtrR gene mutations**
Cephalosporins - mechanisms of resistance

• Modification of target – penicillin binding protein encoded by penA
• Mosaic penA gene fragments from other commensal Neisseria spp
  • Usually found in the throat
  • Naturally less susceptible to cephalosporins
• Specific key amino-acid substitutions in PBP-2

Monitoring AMR

Surveillance programmes

- Monitor trends in resistance
- Monitor drift in susceptibility
- Detect emergence of resistance
- Inform treatment guidelines

Local
National
Regional
Global
GRASP: Gonococcal Resistance to Antimicrobials Surveillance Programme

- Unique enhanced dataset combining epidemiological, behavioural and microbiological information on gonococcal resistance
- Informs treatment guidelines
Percentage of gonococcal isolates resistant to selected antimicrobials: England and Wales, 2000-2017

First line therapy changed from cefixime to ceftriaxone and azithromycin

First line therapy changed from ciprofloxacin to cefixime

Ceftriaxone MIC distribution 2008 to 2017

- Shift in the modal MIC from 0.008 mg/L in 2016 to 0.015 mg/L in 2017
Molecular epidemiology

- Identify associations between sequence types and antimicrobial resistance / sexual orientation, transmission networks
- NG-MAST (*Neisseria Gonorrhoeae* Multi Antigen Sequence Typing)
- Examine variation in two hypervariable genes – *porB* and *TbpB*
- Whole genome sequencing; much greater level of discrimination

Proportion of Genogroup 1407 (cefixime & ciprofloxacin resistant), 2010

<table>
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<td>21-30%</td>
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Associations between NG-MAST Sequence Type (ST) and AMR profile observed

Accounted for most of the decreased susceptibility and resistance to ESCs

* Low numbers of isolates tested
Phylogenetic trees

Compare differences to produce a tree – Single nucleotide polymorphisms (SNP) analysis

A SNP is a position in the genome sequence that differs between genomes – highly related strains have fewer SNPs

Degree of similarity between strains can be used to infer the time since their divergence – split from a common ancestor

Can compare epidemiologically linked cases to the genomic data to confirm linkage (part of a transmission event)

Transmission nomogram

Gonorrhoea treatment failure caused by a *Neisseria gonorrhoeae* strain with combined ceftriaxone and high-level azithromycin resistance, England, February 2018


*Figure 1* Genetic relatedness with previous ceftriaxone resistant isolates of Neisseria gonorrhoeae case imported from Thailand to England, February 2018
Response plans

- Strengthen the surveillance of gonococcal AMR
- Maintain and develop capacity for culture and susceptibility testing
- Increase awareness of appropriate use of antimicrobials
- Establish a strategy for timely treatment failure detection
Thank you

Thank you to Michelle Cole, Gwenda Hughes, and GRASP team