

# Update on NG-MAST



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# Plan of talk



- **Introduction**
- **NG-MAST**
- **Validation of the technique**
- **Clusters of ciprofloxacin resistant gonococci**
- **Epidemiology of gonorrhoea**
- **Medico-legal uses**
- **Future**

# Discriminatory ability



Show sufficient discrimination to **differentiate** between isolates from **unlinked** sources

Sufficiently stable to **identify linked** cases from the **same** source



*Each strain will appear different unless different unless part of a short transmission chain*



# Sequence based typing

- **Examine variation in hypervariable genes**
- **High level of discrimination**
- **High throughput for timely analysis**
- **Unambiguous, manageable, shareable data**

## **NG-MAST**

*Neisseria Gonorrhoeae* **Multi Antigen**  
**Sequence Typing**

# Sequencing internal regions of 2 variable OMPs



*por* gene

**Essential for infection**

**Pore in membrane**

**Immunogenic**

**Epitopes for serotyping**

**Fixed length**

**Trimmed from a conserved  
region**

**490bp variable region**

*tbpB* gene

**Essential for infection**

**Iron acquisition**

**Surface exposed**

**Immunogenic**

**Fixed length**

**Trimmed from a conserved  
region**

**390bp variable region**



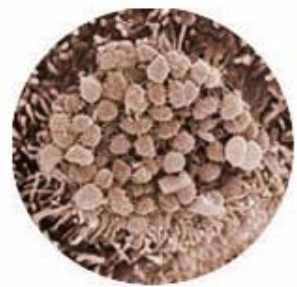
## NG-MAST, for each strain:

- Different *por* and *tbpB* sequence assigned a different allele number
- Each combination of alleles assigned a sequence type (e.g. *por*-02, *tbpB*-05 = ST03)
- Analysis is all web based

[WWW.NG-MAST.NET](http://WWW.NG-MAST.NET)



Three horizontal input fields with rounded ends, currently empty.



### *Neisseria gonorrhoeae*

- ▣ Organism Specific Information
- ▣ *N. gonorrhoeae* Links
- ▣ Download Alleles | STs
- ▣ Batch Query
- ▣ NRDB allele assignment
- ▣ Starting your own NG-MAST study?
- ▣ Contact Curator
- ▣ Administration

#### Profile Query

Please choose—

#### Locus Query

Please choose—

Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Rapid Sequence-Based Identification of Gonococcal Transmission Clusters in a Large Metropolitan Area. *J Infect Dis.* 2004 Apr 15;189(8):1497-1505.

For comments, queries, bugs or suggestions please email [David Aanensen](#)

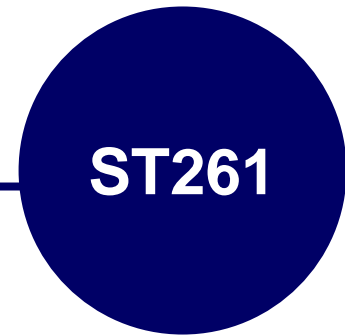


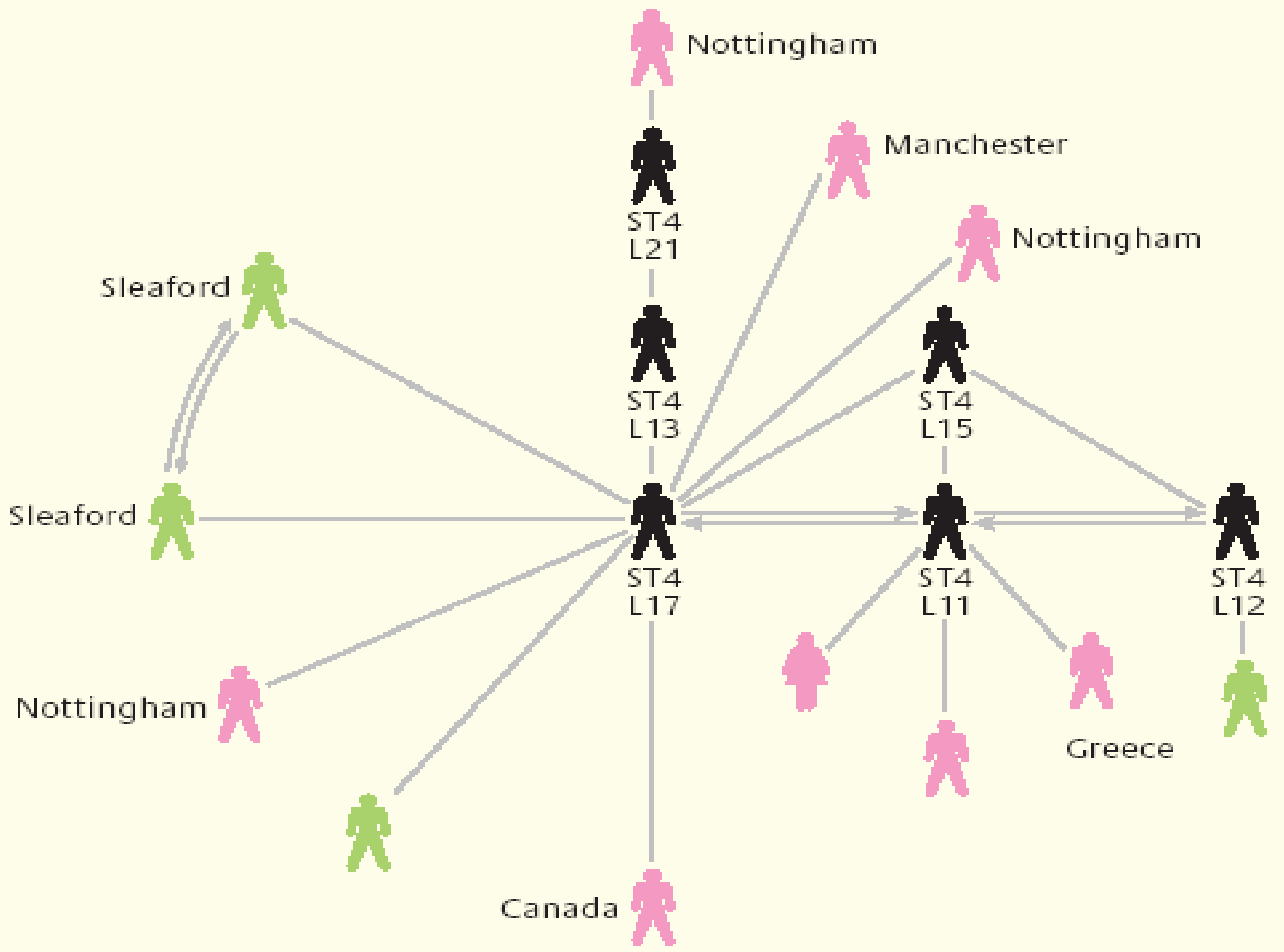
# Validation of NG-MAST



- **Panel of diverse isolates – each different ST.**
- **Ten pairs of isolates from known contacts: –**
  - Indistinguishable within the pair
  - Each pair was distinct.
- **Reproducible:**
  - Same DNA source (80 isolates).
  - New DNA source (20 isolates).
- **Can confirm sexual contact and identify clusters**

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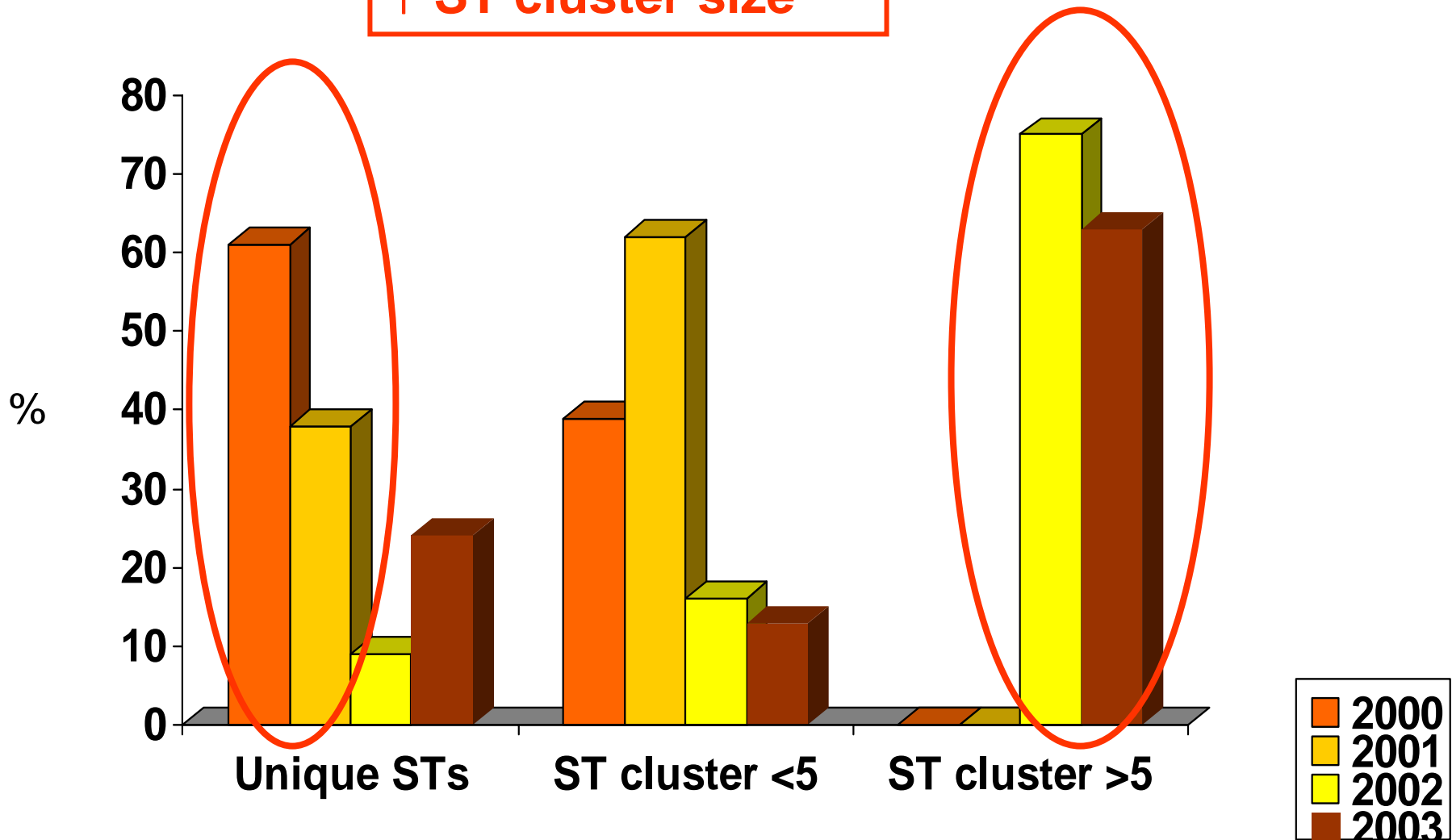




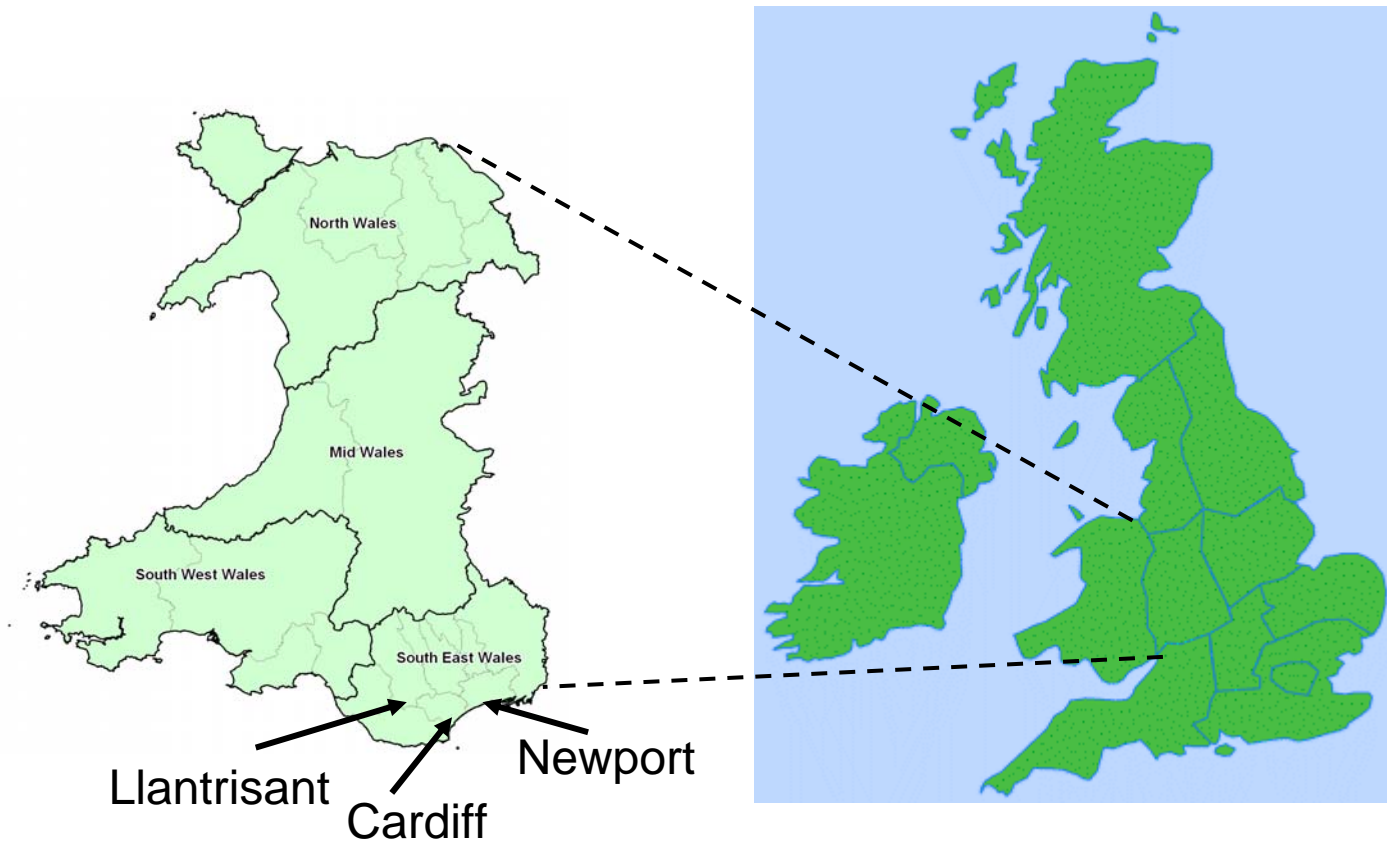
# Ciprofloxacin resistant GC

- **Unemo et al, APIMS 2007;115:231-41**
- **Palmer et al, Int J STD & AIDS 2006;17:254-6**
- **Moodley et al, Sex Transm Dis 2006;33:357-60**
- **Palmer et al, Sex Transm Infect 2005;81:403-7**
- **Martin et al, J Infect Dis 2005;192:1191-5**

↑ Incidence  
↓ Importation  
↑ ST cluster size



# Molecular epidemiology of GC in Wales



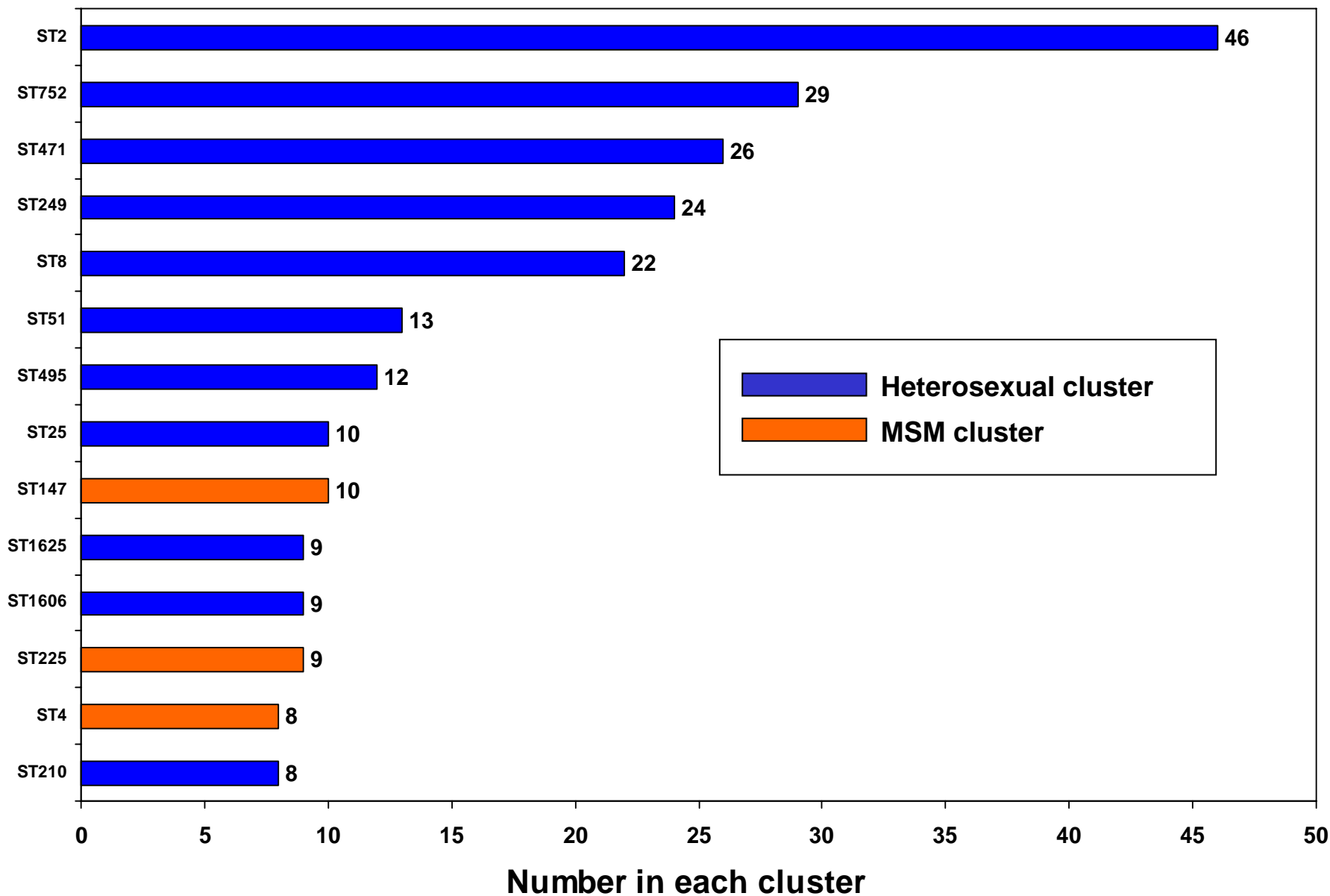
**Cole et al, Abstract No: 400, Code P455 Tuesday 31 July**

## Welsh study



- **540 consecutive isolates over 17 month period**
- **Patients attending 3 clinics, 60% men, 40% women**
- **186 different sequence types (142 por, 68 tbpB alleles)**
- **501 isolates (93%) typed by NG-MAST**
  - 56 clusters of  $\geq 2$  with same ST
  - 130 unique STs
- **Large clusters of  $\geq 8$  could be defined as heterosexual or MSM associated**

# Welsh study: Clusters of 8 or more

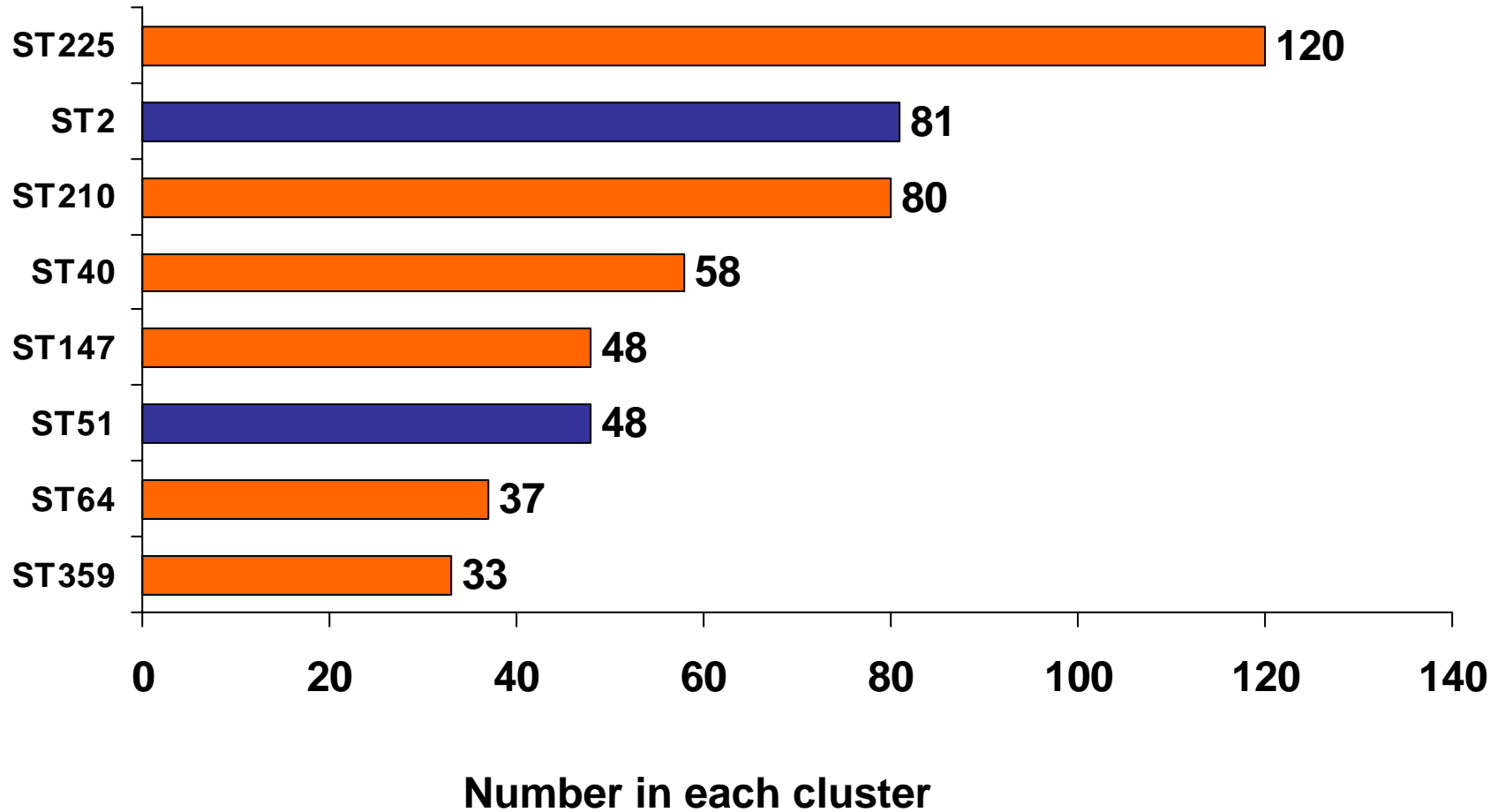


# Molecular epidemiology of GC in London

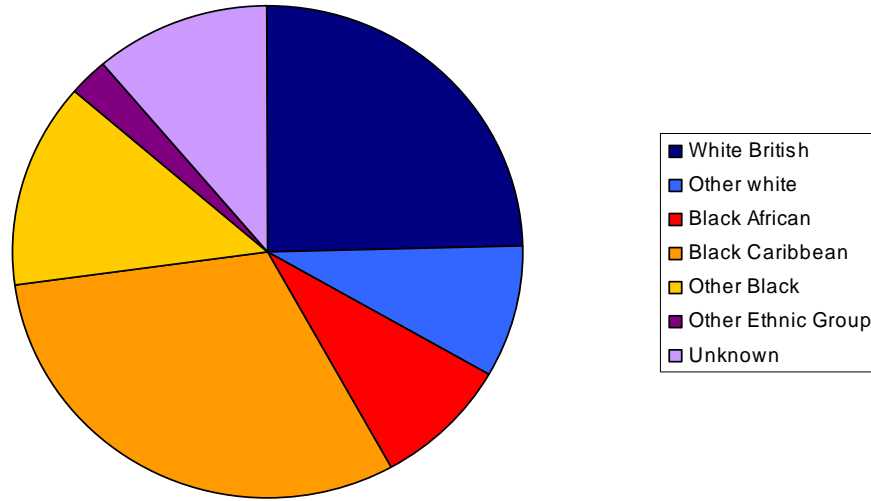


- High prevalence, 45-50% of GC in Eng & Wales
- Large number of GUM clinics, diverse population
- Information on sexual contacts may be incorrect or unknown.
- 2045 isolates collected over 6 months were typed
- 21 prevalent strains, infecting between 20 and 124 individuals.
- 7 were predominantly from MSM, 14 from heterosexuals.

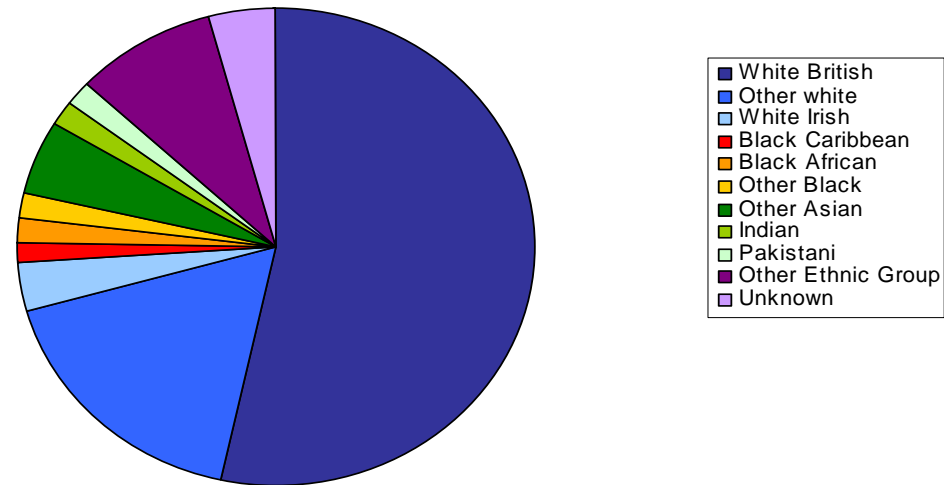
# London study: Major clusters

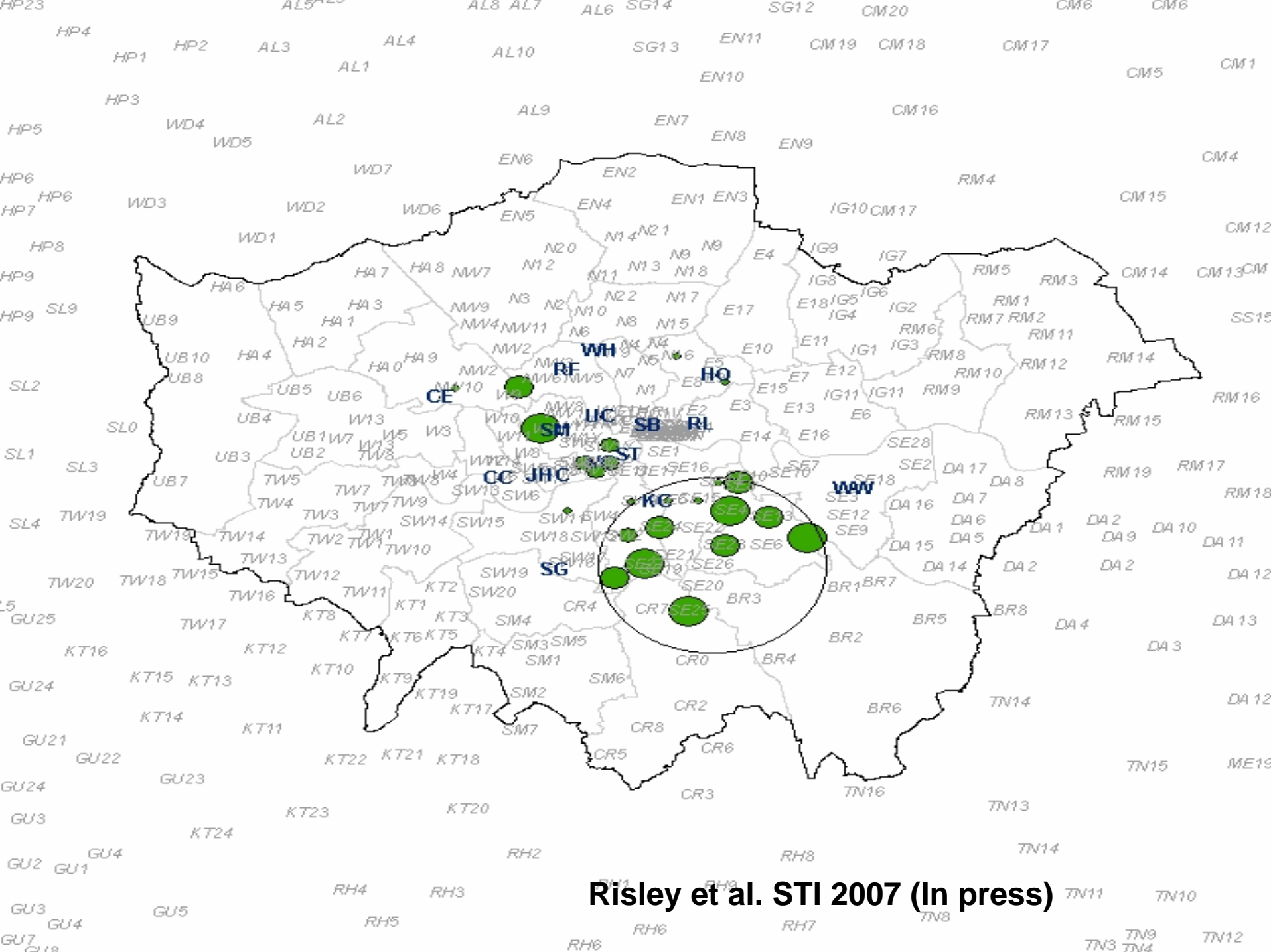


**Ethnicity of ST2**



**Ethnicity of ST225**





Risley et al. STI 2007 (In press)

# Medico-legal case



- **Adult male accused of sexual abuse but refused to provide an invasive sample.**
- **He was required to remove his underpants which were heavily stained from apparent discharge.**
- **Amplification of four gonococcal genes suggests that it is highly likely to have been from a genital infection.**
- **Isolate from child and extract gave ST 403 which was present in the NG-MAST database of 4000 only in two additional isolates.**
- **Perpetrator changed his plea to guilty BEFORE case was brought to trial.**

# Medico-legal case



- **Three sisters infected with GC at multiple sites (8 isolates)**
- **Father also positive for GC**
- **All isolates were confirmed as same ST and indistinguishable by multiple methods**
- **In court father found not guilty because it was not possible to show direction of infection**

# Molecular methods



- **First report using molecular methods for detection and typing of gonococcal DNA from clothing.**
- **Can be achieved if DNA load is sufficient for amplification.**
- **In first case four genes were amplified, two for detection and two for typing.**
- **Decreases the possibility of a non-specific result.**
- **May be necessary to include sequence data.**
- **Not tested in a court of law.**

# Future of NG-MAST



- **As part of the ESSTI programme**
- **Establish an EQA programme**
- **Compare STs in different countries**
- **Compare related sequence types**
- **Compare AmR patterns of STs in different countries**