**Introduction**

- Cases of syphilis continue to rise in NSW, Australia
- 16 cases in 1999; 598 in 2013
- Most cases are in gay and bisexual men; 50% are HIV positive
- Testing and public health campaigns limited effect so far
- Molecular studies can provide important public health information
- Outbreak investigation and disease dynamics

**Syphilis subtypes**

- First subtyping system developed in 1998 by CDC
  - Number of 600bp repeats in Acidic Base Protein (apr gene) - gives a number
  - RFLP analysis of T. pallidum repeat (tpr) sub-family-II genes - gives a letter

**Enhanced subtyping: Strain types**

- Addition of tp0548 gene sequence analysis

**Data from Melbourne**

- No association with HIV status

**Association of strain/subtype with drug resistance?**

- No association found in UK\(^1\) or San Francisco\(^2\)
- Some association in Seattle\(^3\)

**Aim**

- To determine the strain-types of *T. pallidum* in Sydney, Australia, and investigate associations with clinical features, epidemiological information and drug resistant A2058G mutations.
**Methods**

**Setting:** Sydney, Australia

**Samples:** Clinician initiated testing

Stored PCR positive samples for *T. pallidum* Tpp47 from 2004-11

- PCR amplification of *arp* gene and product size determined by electrophoresis
- PCR amplification then RFLP of *tpr* gene - classified as per Pillay 1998
- Sequencing of TP0548 gene and compared with previously described sequences as described by Grillova STD 2015

**Clinical:**

- Reviewed medical records of patients attending one of 4 clinics
  - Sydney Sexual Health Centre, East Sydney Doctors, Holdsworth House Medical Practice, Taylor Square Private Clinic
- Demographic: age, gender, Aboriginality
- Behavioural: gender of partners, recent sex overseas
- Clinical: HIV status, stage of syphilis, evidence of neurosyphilis

**Drug resistance:** results of previous study on prevalence of A2058G mutation conferring macrolide resistance (Read et al J Clin Micro 2014)

**Results**

409 samples were positive for *T. pallidum* DNA by Tpp47 PCR

- 194 produced products on all 3 gene amplifications
- 2 new TP0548 sequences - confirmed by reverse sequencing - designated M & N

**Results: confirmed strain types in 192 samples**

20 different subtypes based on 1998 CDC classification

- 63% subtype 14d (46% - 68% each year)
- More likely to harbour azithromycin resistance 109/121 (90%) vs 59/71 (70%) *p*<0.001
- No relation with HIV status

**Results: Strain-types-2010 enhanced system**

**Results: Distribution of strain types over time**
Clinical/Demographic associations with strain-type

- Comparison of 14d/g to non-14d/g
- 20/41 (48.9%) 14d/g HIV pos vs 23/50 (46%) HIV neg non-14d/g (p=0.55)
- 2/41 (5%) 14d/g reported sex overseas vs 11/50 (22%) non-14d/g (OR 5.5, 95%CI 1.2-26.4 p=0.03)
- 2 cases of neurosyphilis were both 14d/g

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=91</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>90 male (78 known male partners) 1 female</td>
</tr>
<tr>
<td>Aboriginal</td>
<td>0</td>
</tr>
<tr>
<td>Median Age</td>
<td>39 (range 21-63)</td>
</tr>
<tr>
<td>Stage of Disease</td>
<td>Primary 64 Secondary 27</td>
</tr>
<tr>
<td>HIV Status</td>
<td>Negative 48 Positive 43</td>
</tr>
</tbody>
</table>

Strain-type and macrolide resistance

- Overall 159/192 (83%) were known positive for A2058G mutation
- 91/92 (98.9%) 14d/g were resistant vs 68/100 (68.0%) non-14d/g (OR 43.7 95%CI 5.8-327.4 p<0.001)
- 11/11 14b/g, 8/8 14d/n, 7/7 14e/g and 7/7 14e/g were A2058G positive
- Dominant strain-types are predominantly resistant (92% of strains with >5 samples)
- Strain-types with only 1-5 samples tended not to have resistance (35%)
- Suggest sporadic importation of minor strains?
- Numbers too small to determine if particular strain-types acquired resistance with time

Conclusion

- Multiple strain-types circulating in Sydney
- 14d/g dominant across all years
- Non 14d/g strain types were associated with possible overseas acquisition, and the absence of macrolide resistant mutations
- 2 new TP0548 sequences unique to this sample- designated M & N
- No association with HIV status- sexual mixing likely
- TP0548 type "f" strain previously associated with neurosyphilis
- Further work sequencing new Tpr patterns to confirm if genuinely new
- Comparison with other samples from remote Australia and Asia

Thank you

phillip.read@sesiachs.health.nsw.gov.au

Thanks to Laboratory team at CIDM-PH, Westmead Hospital
Dr Samantha Katz & Dr Allan Pillay –US CDC
Partly funded by RACP Research fellowship grant