

TREPONEMA PALLIDUM STRAIN-TYPES IN AUSTRALIA AND ASSOCIATION WITH MACROLIDE RESISTANCE

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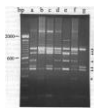
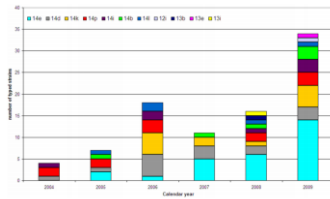
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Introduction

- 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

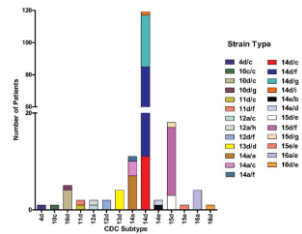
- First subtyping system developed in 1998 by CDC
- Number of 60bp repeats in Acidic Base Protein (apc gene)- gives a number
- RFLP analysis of *T. pallidum* repeat (tpr) sub-family-II genes- gives a letter



Data from Melbourne
 No association with HIV status
 Azzato et al J Clin Micro 2012

Enhanced subtyping: Strain types

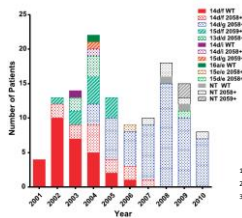
- Addition of tp0548 gene sequence analysis



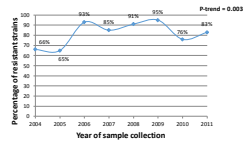
Marra et al JID 2010

Association of strain/subtype with drug resistance?

- No association found in UK¹ or San Francisco²
- Some association in Seattle³



¹Tipple et al STI 2011
²Katz et al STD 2010
³Grimes et al STD 2012



Read P et al J Clin Micro 2014

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

DNA re-extracted and enhanced strain-typing performed (Marra 2010)

- 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 a-l as described by Grillova STD 2015

Methods

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, previous syphilis

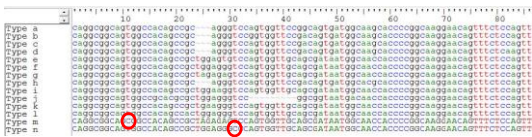
Drug resistance: results of previous study on prevalence of A2058G mutation conferring macrolide resistance (Read et al J Clin Micro 2014)

Results

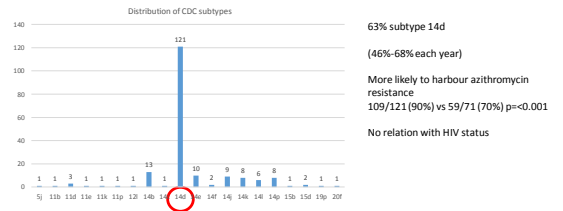
409 samples were positive for *T pallidum* DNA by Tpp47 PCR

- 194 produced products on all 3 gene amplifications
- 2 samples produced new tpr patterns
 - Currently undergoing sequencing for verification
- 2 new TP0548 sequences- confirmed by reverse sequencing- designated M & N

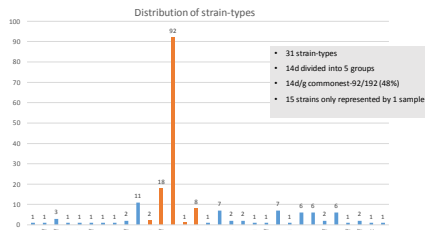
Year	No of samples strain-typed
2004	5
2005	15
2006	2
2007	24
2008	36
2009	34
2010	38
2011	40



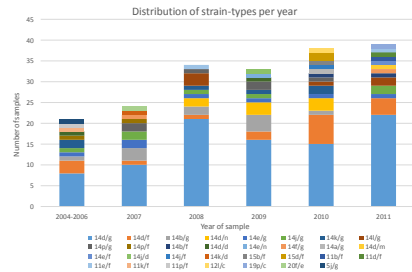
Results: confirmed strain types in 192 samples
20 different subtypes based on 1998 CDC classification



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 (49%) 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/f

Characteristic	N=91
Gender	90 male (78 known male partners) 1 female
Aboriginal	0
Median age	39 (range 21-63)
Stage of disease	Primary 64 Secondary 27
HIV status	Negative 48 Positive 43

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

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