Azithromycin resistance and subtyping of *Treponema pallidum*

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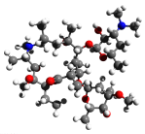
Introduction



Single-Dose Azithromycin versus Penicillin G Benzathine for the Treatment of Early Syphilis

Gabriele Riedner, M.D., Ph.D., Mary Rusizoka, Dipl.Med., Jim Todd, M.Sc., Leonard Maboko, M.D., Michael Hoelscher, M.D., Donan Mmbando, M.D., Eleuter Samky, M.D., Eligius Lyamuya, Ph.D., David Mabey, M.D., Ph.D., Heiner Grosskurth, M.D., Ph.D., and Richard Hayes, D.Sc.

A Randomized, Comparative Pilot Study of Azithromycin Versus Benzathine Penicillin G for Treatment of Early Syphilis

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Azithromycin vs. benzathine penicillin G for early syphilis: a meta-analysis of randomized clinical trials

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N Engl J Med 2005; 353:1236-44 STD 2002 29:486-90 Int J STD AIDS 2008 19: 217-21

Use of azithromycin

- San Francisco
  - 1g for contacts
  - 2g for cases
- British Columbia- Vancouver
  - Mass treatment of >4000 people in 2000
  - Control, then increased
- Appeared as alternative therapy in many guidelines

Reports of resistance

- Street 14 strain of *T.pallidum* has known macrolide resistance mutation A2058G

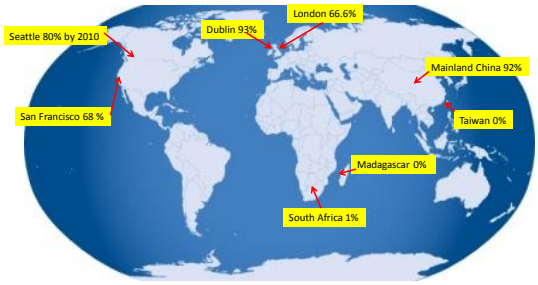
Table 1. The Presence of the 23S rRNA Gene Mutation in *T. pallidum* Samples Collected from Sites in the United States and Ireland from 1912 through 2003.

Geographic Site	Date Sample Collected	Samples with Mutation/ Total Amplifiable Samples
		no./total no. (%)
San Francisco	1999–2002	1/25 (4)
	2003	11/30 (37)
Seattle	2001–2003	3/23 (13)
Baltimore	1998–2000	2/19 (11)
Dublin	2002	15/17 (88)
Historical strains from multiple locations	1912–1987	1/18 (6)

Lukehart et al NEJM 2004 351:154-158

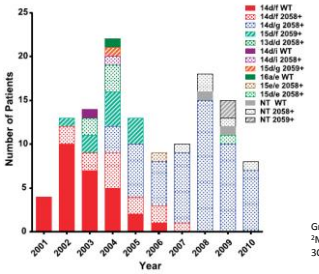
	2002	2003	2004	2005
San Francisco	4%	41%	56%	77%
Seattle	9%	23%	50%	56%

Worldwide reports of macrolide resistance



Reason for increasing resistance?

Selection pressure for *de novo* mutations?
Selection of existing resistant strains?



RR for resistance 2.2 in Seattle[2], and 19.65 in China[3] if receipt of macrolide in prior year

Grimes et al STD 2012 39:954-8
[2]Marra et al JID 2006 194:771-3
3Chen et al Clin Microbiol Infect 2013

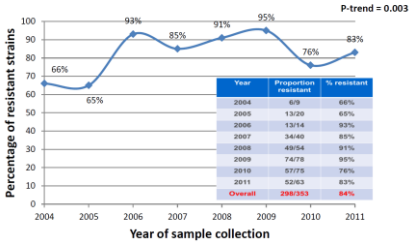
Alternative mutation A2059G

- First reported in Czech republic 2009
  - Treatment failure with spiramycin
  - 14% of samples from Czech republic[1]
- Also:
  - UK 5.6%[2]
  - USA 10-13.2%[3-4]
  - (Taiwan/South Africa 0%)
- NO samples contained A2058G and A2059G

[1]Flasarová et al Acta Derm Venereol 2012, [2]Tipple et al STI 2011, [3]Grimes et al STD 2012, [4]Chen et al J Clin Micro 2013

Azithromycin resistance- Sydney

- Stored samples from 2004-2011 tested for A2058G



Year	Proportion resistant	% resistant
2004	6/9	66%
2005	13/20	65%
2006	13/14	93%
2007	34/40	85%
2008	49/54	91%
2009	74/78	95%
2010	57/75	76%
2011	52/63	83%
Overall	298/353	84%

Read et al. J Clin Micro 2014 52:2776-81

Risk factors for azithromycin resistance

TABLE 2 Factors associated with azithromycin resistance[a]

Factor	Proportion resistant (%)	Univariate OR (95% CI)	*P* value	Multivariate OR[b] (95% CI)	*P* value
Age at diagnosis (yrs)					
≤29	14/15 (93%)	1.0			
30–39	46/54 (85%)	0.41 (0.05–3.57)	0.420		
40–49	81/93 (87%)	0.48 (0.06–4.00)	0.500		
≥50	28/36 (78%)	0.25 (0.03–2.20)	0.212		
Yr of diagnosis					
Pre-2008	30/40 (75%)	1.0			
2008 and onwards	139/158 (88%)	2.44 (1.03–5.77)	0.043	3.20 (1.28–8.07)	0.013
HIV status					
Negative	76/90 (84%)	1.0			
Positive	93/108 (86%)	1.14 (0.52–2.51)	0.741		
Stage of syphilis					
Primary	119/141 (84%)	0.92 (0.32–2.67)	0.885		
Secondary	25/30 (83%)	1.0			
Missing	25/27 (93%)	2.31 (0.51–10.46)	0.277		
Previous syphilis					
No	104/121 (86%)	1.74 (0.51–5.94)	0.371		
Yes	51/59 (86%)	1.82 (0.48–6.94)	0.380		
Missing	14/18 (78%)	1.0			
Sex overseas					
No	149/169 (88%)	1.0			
Yes	20/29 (69%)	0.30 (0.12–0.75)	0.010	0.25 (0.09–0.66)	0.005
Macrolide in last 12 mo					
No	129/155 (83%)	1.0			
Yes	40/43 (93%)	1.22 (0.97–1.54)	0.085	2.82 (0.79–10.05)	0.110

[a] There were 198 subjects for which there were resistance data; results are given for samples where data were available. Data for aboriginality, gender, gender of partner, and neurosyphilis were not analyzed due to small sample sizes.
[b] Multivariate analysis was employed for factors where *P* was <0.2 in the univariate analysis.

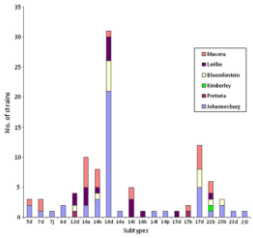
Read et al. J Clin Micro 2014 52:2776-81

T.pallidum subtyping

- Published in 1998
- 1.14Mb 1040 genes- we known what 55% do
- Fully capable of DNA RNA and protein synthesis
- No phosphate uptake, no fatty acid synthesis
- Minimal DNA repair, no nucleotide/amino synthesis
- No electron transport
- Only 22 lipoproteins (vs 132 for *B. burgdorferi)*
- Tpr gene repeats
- In summary: highly adapted obligate parasite

Syphilis subtypes

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



Muller et al STI 2012

Subtype	No of specimens
14*d*	44
14*e*	7
14*j*	3
14*b*	2
14*p*	1
14*k*	1
*e**	2
e†	1
*j**	1
*k**	1
*l**	1
*p**	1
14‡	25§
No results¶	18

Cole et al. STI 2009

Subtypes in Melbourne

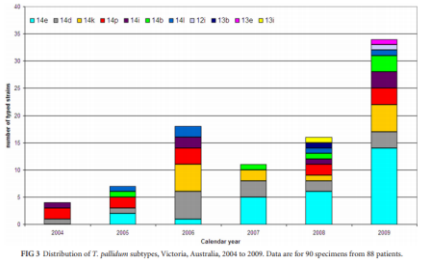
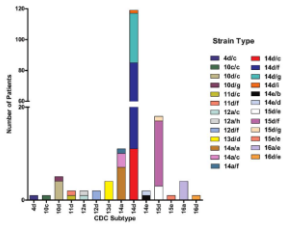


FIG 3 Distribution of *T. pallidum* subtypes, Victoria, Australia, 2004 to 2009. Data are for 90 specimens from 88 patients.

Azzato et al J Clin Micro 2012

Enhanced subtyping

- Addition of tp0548 gene sequence analysis



Marra et al JID 2010

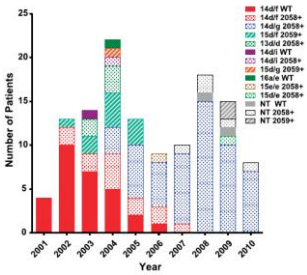
Caveat: one study shows possible variability in same patient- arp and tpr genes

Alternative typing systems- pure sequence based on 23s ribosome, TP0136 and TP0548

Mikalová et al. BMC Microbiology 2013

Association of subtype with drug resistance?

- No association found in UK[1] or San Francisco[2]
- Some association in Seattle[3]



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

Association of subtype with neurosyphilis

- South Africa: Strain 14a associated with 7/13 cases of NS - 14d more common at time[1]
- Seattle: [2]
  - 21/42 (50%) of patients with 14d/f had NS
  - 10/41 (24%) of patients with another strain had NS (p=0.02)
    - (15/42 vs 5/41 had positive CSF VDRL p=0.01)
- Rabbits: subtypes can give different phenotypes, 14d/f causes neuroinvasion[3]

[1]Molepo et al STI 2007, [2]Marra et al JID 2010, [3] Tantalo JID 2005

Conclusion

- Azithromycin resistance is common in resource-rich settings, including Australia
- Subtyping has utility in tracking epidemics, identifying clusters
- Association with neurosyphilis and clinical utility of subtype regarding remains to be determined

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