



**NATIONAL HEALTH  
LABORATORY SERVICE**



***TREPONEMA PALLIDUM:*  
MACROLIDE RESISTANCE AND MOLECULAR SUBTYPING  
OF STRAINS FROM SOUTH AFRICA**

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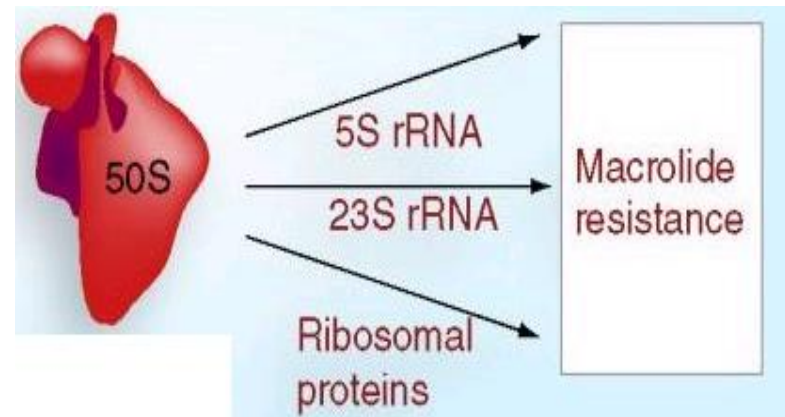
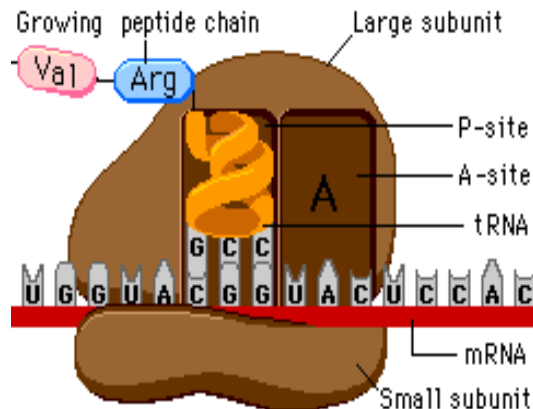
# Introduction

- ***Treponema pallidum* is a non-cultivable spirochaete, and the causative agent of syphilis which is endemic in South Africa**
- **Since syphilis cannot be cultivated, antimicrobial testing is impossible**
- **South Africa: 3 dose regimen of intramuscular benzathine penicillin**
- **Alternatives: oral doxycycline/tetracycline OR a macrolide e.g. erythromycin/azithromycin - clinical failure of azithromycin**



# Introduction

- **Macrolides block protein synthesis by binding reversibly with 23S ribosomal RNA (rRNA) subunit of bacterial ribosomes**
- **A → G mutation (position 2058) in peptidyl transferase region of 23S rRNA identified**
- **Resistance increased from 4-9% (2002) to 56-76.5% (2005) in USA**
- **No data on macrolide resistance in South Africa yet**



# Aim

- **To determine whether the main 23S rRNA mutation (A2058G) that confers macrolide resistance in *Treponema pallidum* is present among DNA obtained from syphilitic ulcers from men presenting to primary healthcare clinics in South Africa**
- **To determine the strain subtype distribution using molecular methods**

# Methodology

- **Specimens: 2005-2009**
  - **TP DNA (+) swab specimens from GUD patients of the STI National Microbiological Surveillance (NMS) program in Gauteng, Northern Cape and Free State as well as GUD specimens collected for a HSV episodic acyclovir treatment trial (HSV4294)**
  - **Ethical approval obtained & participant's consent**
  
- **Processing**
  - **Original genomic DNA used to screen GUD specimens for ulcer causing organisms : multiplex real-time PCR**
  - **All positives were re-tested with commercial real-time PCR kit**

# Methodology

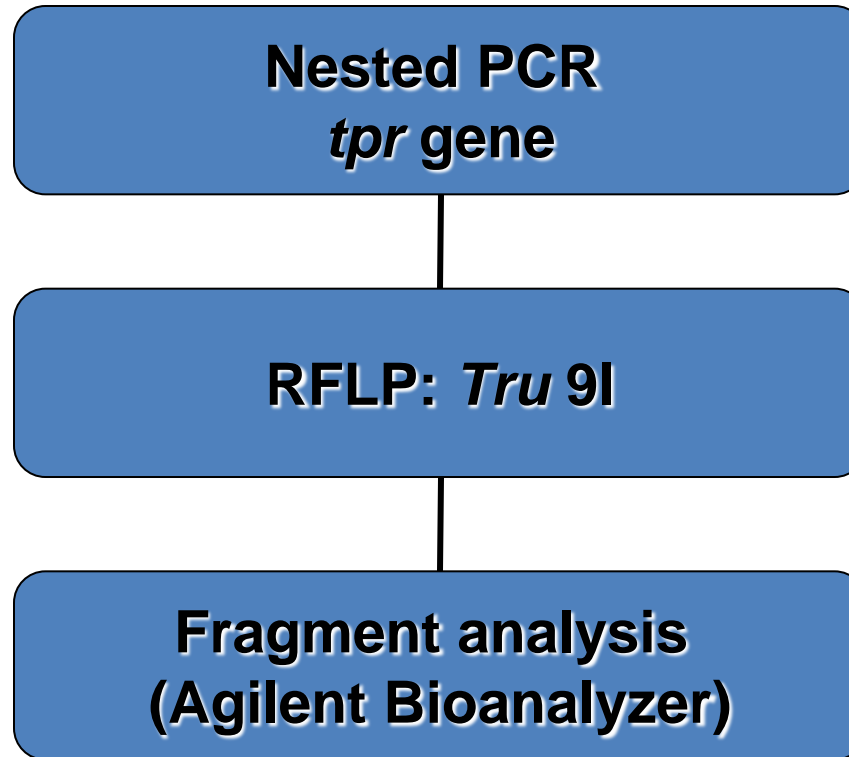
- **Macrolide resistance of *T. pallidum* strains**
  - **These positive samples were screened for the A2058G point mutation using a rapid PCR-based restriction digest assay (Lukehart *et al*, 2004)**
  - **Digestion products separated = samples without mutation (a single band at 628 bp) + samples with mutation (2 restriction fragments 440 and 188 bp)**
  - **Samples producing the mutation fragment would then be sequenced to confirm the presence of the A → G point mutation.**

# Methodology

- **Sub-typing of *T. pallidum* strains: 2 genes**
  - **Acidic repeat protein (*arp*) gene**
  - ***T. pallidum* repeat (*tpr*) gene**
  
- ***arp* gene**
  - **Determine the number of 60 bp tandem repeats**
  - **Touchdown PCR → fragments of various sizes amplified, depending on the number of repeats in the *arp* gene**
  - **Result analysis on Agilent BioAnalyzer**
  - **The number of *arp* gene short tandem repeats estimated by comparison with the amplicon size for the Street 14 strain of *T. pallidum* at 1155 bp, which corresponds to 14 repeats**

# Methodology

➤ *tpr* gene



- Subtypes determined by combining results from *arp* sizing and the *tpr* RFLP pattern

# Results

**Table 1: Study sites and no. of TP (+) cases included in study**

PROVINCE	STUDY	YEAR	No. of ulcer Pts	No. of TP (+) cases	
				Overall	In this study
Gauteng	HSV EpSt	2005-2006	615	30	20
Northern Cape	NMS	2006	25	5	1
Gauteng	NMS	2007	73	5	4
Gauteng	NMS	2008	237	11	11
Gauteng	NMS	2009	323	12	12
Free State	NMS	2007-2008	63	12	12
<b>TOTAL</b>	<b>All Studies</b>	<b>2005-2009</b>	<b>1336</b>	<b>75</b>	<b>60</b>

HSV EpSt = episodic acyclovir therapy



# Results

- **Macrolide Resistance testing**
  - **Restriction-digestion patterns for all 60 samples obtained**
  - **All yielded 628 bp fragment → No A2058G point mutation**
  
- **Subtype distribution**
  - **All 60 TP (+) samples were positive by both *arp* and *tpr* assays and produced full subtype in all cases**

# Results

- 8 *arp* repeat sizes: 5, 7, 8, 12, 14, 17, 22 & 23
- 8 *tpr* RFLP patterns: a, b, d, e, l, i, j & p
- Common subtypes: 14d(43%), 17d(13%), 14b(7%), 22b(5%), 23b(5%)
- Combined 17 subtypes: 12 subtypes among 28 specimens from Gauteng NMS, 5 subtypes in HSV Episodic study (Gauteng) and 6 subtypes among 12 specimens from Free State NMS, 1 subtype from Northern Cape

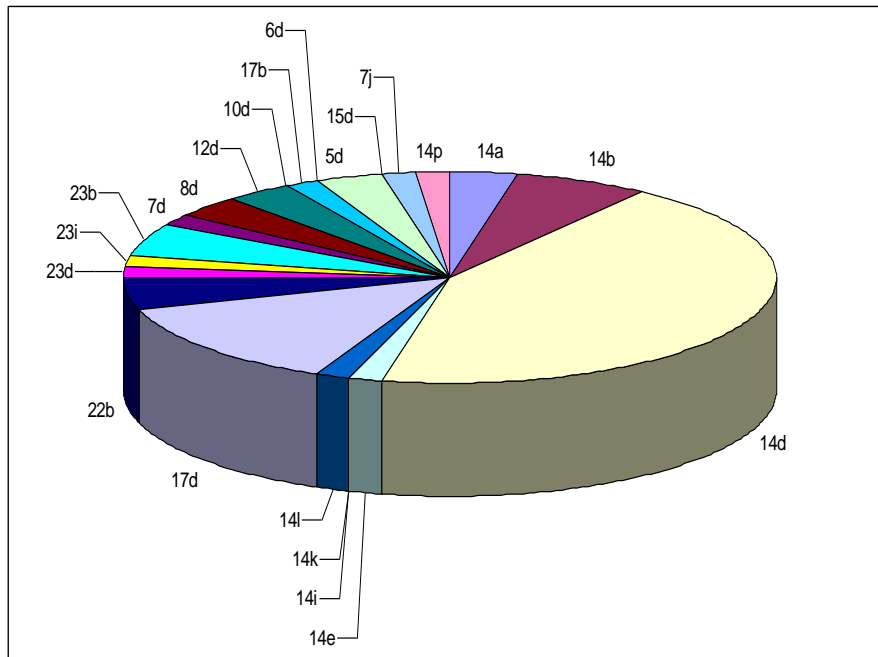


Figure 1: Subtype distribution of strains

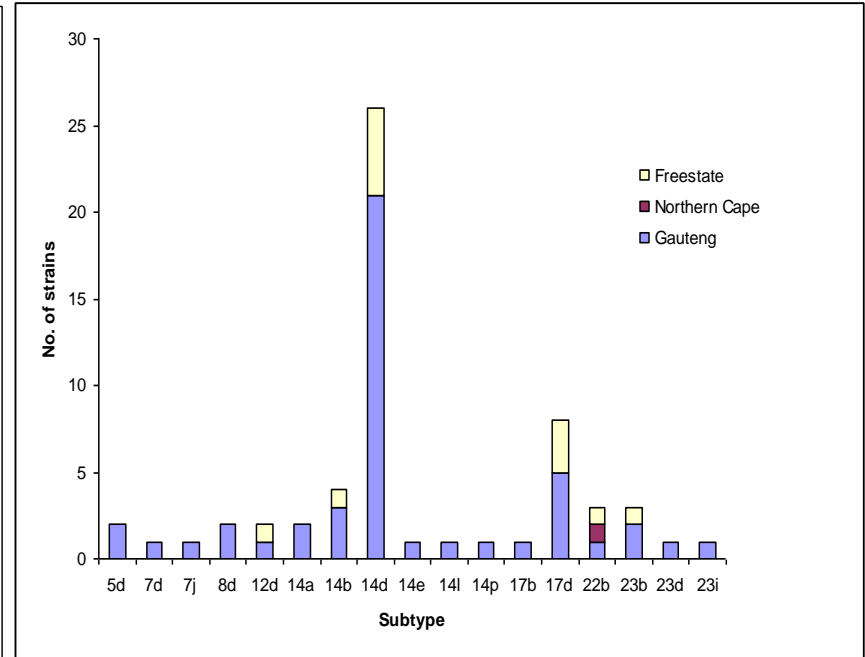


Figure 2: Distribution of *T. pallidum* subtypes by region

# Limitations

- **We were not able to include all of the previously positive *T. pallidum* specimens from our surveys, most likely as a result of DNA degradation over time**
- **The geographical distribution of the samples was not evenly spread among the cities where studies were undertaken as evidenced by the fact that more than half of our specimens came from Johannesburg**
- **We did not have the required data to epidemiologically link syphilis cases in the genital ulcer aetiological surveys**

# Discussion

- **Syphilis was first introduced to SA in the 17<sup>th</sup> century and has since taken on epidemic proportions worldwide**
- **Decline due to intervention studies and management of STIs at public health facilities**
- **This was the first study in SA to embark on examining both macrolide resistance profiles and sub-type distribution of *T. pallidum* strains to understand the molecular epidemiology of syphilis**
- **The point mutation responsible for macrolide resistance has not been detected in South Africa**

# Discussion

- **Although macrolide resistance is unreported in Africa, it could emerge through drug pressure or importation in the future**
- **Most prevalent *T. pallidum* subtype in South Africa is 14d**
- **The arp and tpr gene subtyping system is a useful tool to study epidemiological related strains**
- **Surveillance should be implemented in a bigger sample size and over a longer time to determine trends**

# Aknowledgements

- **Dr Sheila Lukehart, University of Washington, Seattle: providing macrolide resistant *T. pallidum* Street 14 strain (control DNA)**
- **Dr Allan Pillay, CDC: amended *T. pallidum* sub-typing protocol**
- **Gabriela Paz-Bailey, Centre for Health Studies, Guatemala : HSV episodic study isolates**
- **NHLSRT: funding**
- **NICD-STIRC staff**