

New diagnostics for syphilis and yaws and detection of *Haemophilus ducreyi* in cutaneous lesions in children

Allan Pillay

Laboratory Reference & Research Branch,
Division of STD Prevention, Centers for Disease Control & Prevention, Atlanta, USA



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Overview

- "Proof of concept" for use of azithromycin for MDA in yaws endemic countries
- A molecular diagnostic assay that distinguishes between the 3 *T. pallidum* subsp.
- Field evaluation of the Chembio DPP Screen & Confirm Assay for yaws diagnosis
- Testing for other etiologic agents in cutaneous lesions in children (*Haemophilus ducreyi*, *Mycobacterium ulcerans*)
- WGS and susceptibility testing of *H. ducreyi* isolates



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Background

- WHO initiated the yaws eradication program in 2012
- Single oral dose azithromycin was efficacious as benzathine penicillin for yaws treatment - Mitjà O. et al. *Lancet* 2012
- Diagnosis of active yaws case by PCR/sequencing – Pillay et al. *JCM* 2011
- Pilot studies to assess the prevalence of yaws and efficacy of azithromycin were done in several countries
 - Papua New Guinea
 - Solomon Islands
 - Vanuatu
 - Ghana



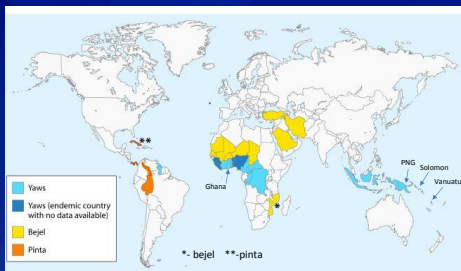
Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

- Yaws is caused by *T. pallidum* subsp. *pertenue*
- Spread by skin-to-skin contact mainly among children
- 3 *T. pallidum* subsp.: *pertenue*, *pallidum* (syphilis), *endemicum* (bejel)
 - Cannot be cultivated on routine laboratory media
 - Very closely related genetically and antigenically
 - Indistinguishable on the basis of morphology or serology
 - Differences in mode of transmission, geographic distribution, invasion of CNS, and infection of the fetus
- *T. pallidum* subsp. could be differentiated by using a combination of methods including PCR, sequencing, and/or RFLP analysis



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Current geographical distribution of endemic treponematoses



South-East Asia: Indonesia and Timor-Leste
Pacific region: Papua New Guinea, Solomon Islands, Vanuatu
Africa: Benin, Cameroon, Central African Republic, Congo, Côte d'Ivoire, DRC, Ghana, Togo



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Objectives

- Develop/Evaluate a real-time PCR assay that can differentiate between *T. pallidum* subsp. *pallidum* (syphilis), subsp. *endemicum* (bejel), subsp. *pertenue* (yaws)
- Evaluate the DPP Screen & Confirm assay versus TPPA & RPR
- Assess the prevalence of yaws in Vanuatu and Ghana before and after mass drug administration with a 30 mg/kg dose of azithromycin




Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Methods

- **Study population**
 - Children (ages 5- 14) with clinically suspected yaws lesions on Tanna Island, Vanuatu, and West Akyem Municipality, Ghana
 - Participants were randomly selected from primary schools, villages, & Health Centers on Tanna & primary schools in Ghana
 - Total community treatment with 30 mg/kg azithromycin

- **Sample collection pre- and post-MDA**
 - Finger prick for RPOCT
 - Blood for serology
 - Lesion swabs in AssayAssure transport medium for PCR
 - No samples from Vanuatu post-MDA




Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention


- **Serologic testing**
 - RPR (quantitative), TPPA
 - Chembio DPP Screen & Confirm Assay - immunochromatographic

Trep/Non-trep


Non-Reactive
Control Line Only



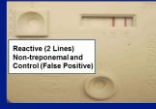
Confirmed Reactive
3 Lines




Reactive (2 Lines)
Treponemal and Control (Old or treated cases)




Reactive (2 Lines)
Non-treponemal and Control (False Positive)





Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

- **Molecular testing**
 - TaqMan-based real-time multiplex PCR to differentiate among the 3 *T. pallidum* subsp. *Chi et al. Am. J. Trop. Med. Hyg. Jan 2015*
 - Targets - *T. pallidum* subsp. *pallidum* & subsp. *endemicum* (two regions of *tpri*, *tp0620*), *T. pallidum* subsp. *pertenue* (*tp0858*); and an internal control (human *RNaseP*)
 - Confirmation of *T. p.* subsp. *pertenue* – PCR/sequencing of an intergenic spacer (IGR19) and a segment of *tpri*
 - Real-time triplex PCR - Detection of 23S rRNA point mutations associated with azithromycin resistance (A2058G, A2059G, Wild Type) *Chen et al. JCM. 2013*
 - Real-time duplex PCR: Detection of *H. ducreyi* and *M. ulcerans* (buruli ulcer)




Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Serology/PCR Results


	Vanuatu		Ghana	
	Pre-MDA	Post-MDA	Pre-MDA	Post-MDA
TPPA & RPR	35.8% (63/176)	18.6% (16/43)	33.6% (109/321)	6.5% (3/46)
PCR <i>T. pertenuae</i>	14.9% (27/181)	ND	17.3% (31/179)	0/49
PCR <i>H. ducreyi</i>	40.3% (73/181)	ND	27.4% (49/179)	28.6% (14/49)
<i>T. pertenuae</i> / <i>H. ducreyi</i>	6	ND	7	0
23S rRNA AzR mutations	0	ND	0	0
<i>M. ulcerans</i>	0	ND	0	0

R1/R4



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

- Detection of *T. pertenuae*-specific DNA from skin lesions was associated with dual RPR/TPPA seropositivity
- Samples with RPR titers \geq R4 were more likely to be *T. pertenuae* PCR-positive




Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Performance Characteristics of the Non-treponemal Line of the DPP-POC Test in Cases Clinically Diagnosed as Yaws (Ghana and Vanuatu)

		RPR		Total
		Positive	Negative	
DPP Non-treponemal Line	Positive	145	17	162
	Negative	26	305	331
		171	322	493

Sensitivity = 84.8%
Specificity = 94.7%

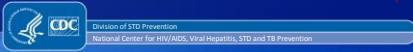


Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Performance Characteristics of the Treponem Line of the DPP-POC Test in Cases Clinically Diagnosed as Yaws (Ghana and Vanuatu)

		TP-PA		
		Positive	Negative	Total
DPP Treponemal Line	Positive	180	50	230
	Negative	24	239	263
		204	289	493

Sensitivity = 88.2%
Specificity = 82.7%

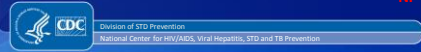


Performance of DPP-POC Test in Cases Clinically Diagnosed as Yaws by *T. pertenuis* PCR Test Result (Ghana and Vanuatu)

		TP-PCR		
		Positive	Negative	Total
DPP Dually Reactive	Positive	44	56	100
	Negative	7	206	213
		51	262	313

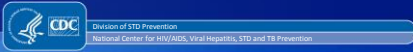
Sensitivity = 86.3%
Specificity = 78.6%

PPV = 44.0%
NPV = 96.7%



Characterization of *H. ducreyi* isolates

- Isolated 5 strains in Vanuatu and 2 in Ghana
- Vanuatu strains identified by 16S rDNA sequencing in Sydney
- MICs for azithromycin, ceftriaxone, and penicillin determined by E-test
- Biochemical testing and antimicrobial susceptibility testing using the agar dilution method was done at CDC
- Whole genome sequencing of Vanuatu and Ghana isolates

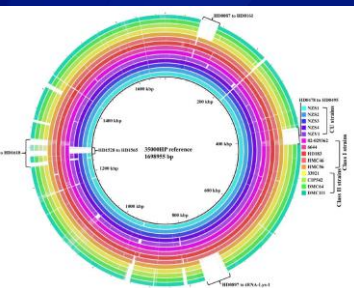


MICs of cutaneous and genital ulcer strains

Strain	Class	AMX	PEN	AMC	CRO	DOX	GP	AZT	ERY
NZ51	CU	0.5	0.25	0.5	0.008	<0.125	0.15	0.015	0.125
NZ52	CU	1	0.25	1	0.008	0.5	0.015	0.03	0.125
NZ53	CU	1	0.25	1	0.015	0.5	0.015	0.03	0.125
NZ54	CU	1	0.25	1	0.015	0.5	0.015	0.03	0.125
NZV1	CU	1	0.25	1	0.008	0.5	0.015	0.03	0.06
VA85	CU	1	0.25	1	0.008	0.25	0.015	0.03	0.125
VA86	CU	1	0.25	1	<0.002	<0.125	0.008	0.03	0.06
VA103	CU	0.5	0.25	1	0.008	0.5	0.015	0.03	0.06
VA171	CU	0.5	0.25	1	0.008	0.5	0.015	0.03	0.06
VA168	CU	1	0.25	1	0.008	0.5	0.015	0.03	0.06
GHD44	CU	0.5	0.125	1	0.008	0.25	0.008	0.03	0.06
GHD47	CU	0.5	0.125	1	0.008	0.25	0.008	0.03	0.06
35000HP	Class I	1	1	2	0.03	0.25	0.008	0.03	0.125
82-029362	Class I	2	1	4	0.008	8 TM	0.015	0.015	0.015
6644	Class I	128 ^R	>256 ^R	4	0.004	16 TM	0.015	0.015	0.015
HD183	Class I	64 ^R	>256 ^R	4	0.008	8 TM	0.002	0.03	0.125
HMC46	Class II	128 ^R	>256 ^R	4	0.008	4 TM	0.015	0.03	0.015
HMC56	Class II	>256 ^R	>256 ^R	8	0.008	4 TM	0.015	0.03	0.015
DMC64	Class II	>256 ^R	>256 ^R	4	0.004	8 TM	0.008	0.03	0.06
DMC111	Class II	>256 ^R	>256 ^R	4	0.004	0.25	0.008	0.06	0.06
CIP42	Class II	0.5	0.25	1	0.004	0.25	0.004	0.03	0.06
33921	Class II	256 ^R	>256 ^R	4	<0.002	16 TM	0.002	0.06	0.125

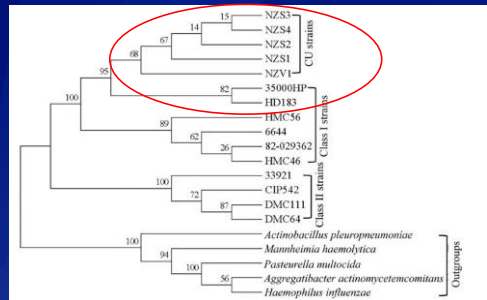
E-test:
PEN & AZM – same
CRO – differed by 2 dil

Adapted from Gangaiah et al. PLoS NTDs, July 2015



- CU strains - no additional genes
- CU strains have all known virulence genes
- 20kb deletion in CU strains
- Class II strains – 3 major deletions

Gangaiah et al. PLoS NTDs, July 2015



Gangaiah et al. PLoS NTDs, July 2015



Azithromycin dosing study - Ghana



HD PCR +
TPE PCR -
DPP -
30 mg/kg
AZM

02/06/2015 14:38



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

4-week follow-up



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention



TPE PCR +



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention



HD PCR +
TPE PCR +

7/06/2015 14:01

Summary

- No PCR-positive yaws cases identified in Ghana post MDA
- Azithromycin is effective for treatment of yaws and MDA was a success in Ghana
- Real-time PCR assays were useful for confirmation of a yaws diagnosis, screening for molecular markers for azithromycin resistance, and detection of cutaneous *H. ducreyi*
- DPP Screen & Confirm Assay is a useful screening test to exclude yaws in cases with a high index of suspicion on clinical grounds
- Higher proportion of low-titer ($\leq 1:2$) RPR positivity was found in Vanuatu vs Ghana



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

- Programs should aware about the variability of performance of DPP, associated with low-titer RPR positivity, and take this into account for surveillance, monitoring impact and detection of infectious cases
- *H. ducreyi* is a significant cause of cutaneous lesions in yaws-endemic countries: Ghana, Vanuatu, PNG, Solomon Islands (32% pre- 35% post-MDA)
- MDA with azithromycin had limited impact on cutaneous lesions caused by *H. ducreyi*
- 45% of children with lesions in Vanuatu and 55% in Ghana were undiagnosed



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Acknowledgment

<p>CDC LRRB Cheng Chen Kai-Hua Chi Samantha Katz Damien Danavall Eli Nachamkin</p> <p>WHO Kingsley Asiedu Jakob Kool</p>	<p>CDC CGH Ye Tun Ron Ballard</p> <p>Country/MOH Vanuatu: Fasiah Taleo David Fegan</p> <p>Ghana: Shirley Simpson Cynthia Kwakye Kwasi Addo</p>	<p>Monica Lahra The Prince of Wales Hospital, Sydney</p> <p>Stanley Spinola, Indiana University, USA</p>
--	--	--



Division of STD Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention

Save the Date!
2016 STD Prevention Conference

Atlanta, GA | September 2016
www.cdc.gov/stdconference/

