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

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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* subsps.
- Field evaluation of the Chembio DPP Screen & Confimm 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

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

Objectives

- Develop/Evaluate a real-time PCR assay that can differentiate between *T. pallidum* subsps. *pallidum* (syphilis), *endemicum* (bejel), *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

Current geographical distribution of endemic treponematoses
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

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

- **Molecular testing**
  - TaqMan-based real-time multiplex PCR to differentiate among the 3 T. pallidum subs.
    - **Targets** - T. pallidum subsp. pallidum & subsp. endemicum (two regions of tpr, tpo620), T. pallidum subsp. pertenue (tple827), and an internal control (human RNase)
    - Confirmation of T. p. subs. pertenue – PCR/sequencing of an intergenic space (IGR19) and a segment of tpr1
  - Real-time triplex PCR - Detection of 23S rRNA point mutations associated with azithromycin resistance (A2058G, A2059G, Wild Type)
    - Chien et al. JCM. 2013
  - Real-time duplex PCR: Detection of H. ducreyi and M. ulcerans (buruli ulcer)

**Serology/PCR Results**

<table>
<thead>
<tr>
<th></th>
<th>Vanuatu</th>
<th>Ghana</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-MDA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. pallidum</td>
<td>35.8% (63/176)</td>
<td>18.6% (16/143)</td>
</tr>
<tr>
<td>TPPA &amp; RPR</td>
<td>33.6% (100/301)</td>
<td>6.5% (3/46)</td>
</tr>
<tr>
<td>PCR T. pertenue</td>
<td>14.9% (27/181)</td>
<td>ND</td>
</tr>
<tr>
<td>PCR H. ducreyi</td>
<td>74.3% (132/179)</td>
<td>ND</td>
</tr>
<tr>
<td>PCR M. ulcerans</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>23S rRNA AzR mutations</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Detection of T. pertenue-specific DNA from skin lesions was associated with dual RPR/TPPA seropositivity**

**Samples with RPR titers ≥ R4 were more likely to be T. pertenue PCR-positive**

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

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPP Non-treponemal Line</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>145</td>
<td>17</td>
<td>162</td>
</tr>
<tr>
<td>Negative</td>
<td>26</td>
<td>305</td>
<td>331</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>64.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
<td>94.7%</td>
<td></td>
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**Sensitivity = 64.6%**

**Specificity = 94.7%**
Isolated 5 strains in Vanuatu and 2 in Ghana

Biochemical testing and antimicrobial susceptibility testing using the agar dilution method was done at CDC.

Whole genome sequencing of Vanuatu strains identified by 16S rDNA sequencing.

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

<table>
<thead>
<tr>
<th>DPP Treponemal Line</th>
<th>TP-PA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>180</td>
<td>50</td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td>239</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>204</td>
<td>289</td>
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Sensitivity = 88.2%
Specificity = 82.7%

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

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<tr>
<td>Positive</td>
<td>44</td>
<td>56</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>206</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51</td>
<td>262</td>
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Sensitivity = 88.3%
Specificity = 78.6%

PPV = 44.0%
NPV = 96.7%

**MICs of cutaneous and genital ulcer strains**

- **E-test:** PEN & ADM - same
- CRO - differed by 2-dils

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Azithromycin dosing study - Ghana

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

4-week follow-up

- HD PCR + TPE PCR +

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 (≤ 1:2) RPR positivity was found in Vanuatu vs Ghana

- 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
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WHO
Kingsley Asiedu
Jakob Kool

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