Emergence of Pediatric Melioidosis in Siem Reap, Cambodia

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Melioidosis

Organism: *Burkholderia pseudomallei*

- Aerobic, motile, GNB
- Soil and water saprophyte
- Endemic areas: Northern Australia and Southeast Asia
- Clinical manifestations can be varied
- Time of standard treatment:
  - Intensive phase: 2-3 weeks (ceftazidime)
  - Oral treatment (TMP-SMX, Doxy): 12-20 weeks
- High mortality rate

Melioidosis in children

- Childhood infection in northeast Thailand accounts for around 10% of cases.

- Acute suppurative parotitis accounts for one-third of pediatric cases.

(Dance DA, et al., 1989)
Melioidosis is under-diagnosed in Cambodia:

i) laboratories are not equipped to grow the bacterium

ii) clinicians are not yet familiar with melioidosis
Antibodies to *Burkholderia pseudomallei* were detected in 16% of Children in Siem Reap

B. *pseudomallei* was isolated from 30% of soil samples ranged from 1-5,000 (median 90 CFU/g, IQR 20-250CFU/g of soil)

**Indirect hemagglutination assay titer for 968 children**

**Antibodies to *Burkholderia pseudomallei* were detected in 16% of Children in Siem Reap**
Aim of study

To report 39 cases of melioidosis at the Angkor hospital for Children (AHC) in Siem Reap, the first reported cases in Cambodian children.
Angkor Hospital for Children (AHC) serves as the paediatric department for Siem Reap’s Provincial Hospital since 1999. Currently the outpatient department sees 400-450 children each day and maintains 50 inpatient beds.
Materials and Methods

- Study period: October 2005 and December 2008 (N= 2,235 patients)
- Microbiology methods: culture
- Demography and clinical information
- Genotyped using multilocus sequence typing (MLST)
  - 39 invasive isolates (in this study)
  - 14 soil isolates (previously reported; Wuthiekanun V, et al., 2008)
Microbiological methods

Identifications
- Gram stain, oxidase test
- Colistin and gentamicin disc screening test
- Latex agglutination test
- API 20NE profile

Susceptibility testing
Bacterial genotyping

Multi-Locus Sequence Typing (MLST)

CHROMOSOMAL DNA

AMPLIFY ~ 450-BP INTERNAL FRAGMENTS OF SEVEN HOUSE-KEEPING GENES

SEQUENCE THE SEVEN GENE FRAGMENTS ON BOTH DIRECTIONS

COMPARE THE SEQUENCES OF EACH GENE FRAGMENT WITH THE KNOWN ALLELES AT THE LOCUS

ASSIGN ALLELES AT THE SEVEN LOCI TO GIVE THE ALLELIC PROFILE

COMPARE THE ALLELIC PROFILES, AND OBTAIN SEQUENCE TYPE (www.mlst.net)
Summary data for 39 children with melioidosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>15</td>
<td>38%</td>
</tr>
<tr>
<td>Age (yrs), median</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Ranges, yrs</td>
<td>1.6-16.2</td>
<td></td>
</tr>
<tr>
<td>Interquartile range (IQR)</td>
<td>4.1-12.4</td>
<td></td>
</tr>
<tr>
<td>Source of isolate;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Blood</td>
<td>9</td>
<td>23%</td>
</tr>
<tr>
<td>- Pus</td>
<td>29</td>
<td>74%</td>
</tr>
<tr>
<td>- Respiratory secretion</td>
<td>1</td>
<td>3%</td>
</tr>
<tr>
<td>Severity of infection;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Localized</td>
<td>27</td>
<td>69%</td>
</tr>
<tr>
<td>- Disseminated</td>
<td>12</td>
<td>31%</td>
</tr>
<tr>
<td>Died during admission</td>
<td>8</td>
<td>21%</td>
</tr>
<tr>
<td>Time to death (days), median</td>
<td>2</td>
<td>Range; 0-23 days</td>
</tr>
</tbody>
</table>
Demographic results

39 cases of culture-proven melioidosis were identified between Oct 2005 and Dec 2008 (2005 (3 months), n=2; 2006, n=9; 2007, n=13; 2008, n=14)
Result of Multi Locus Sequence Typing

Neighbor-joining tree using concatenated sequence of all 7 loci for Cambodian isolates

N = 39

N = 14

Neighbor-joining tree using concatenated sequence of all 7 loci for Cambodian isolates ([www.mlst.net](http://www.mlst.net))
Result of Multi Locus Sequence Typing

Neighbor-joining tree using concatenated sequence of all 7 loci for Cambodian isolates together with Thailand isolates which downloaded from the MLST website (www.mlst.net)

Neighbor-joining tree using concatenated sequence of all 7 loci for Cambodian isolates together with Thailand isolates which downloaded from the MLST website (www.mlst.net)
Discussions

- This is the first description of pediatric melioidosis in Cambodia.

- Many of the isolates responsible were defined as novel STs, but Cambodian isolates from soil and invasive were highly related to a collection of isolates in nearby Thailand.

- 39 cases are likely to represent the tip of the iceberg since diagnosis relies on microbial culture, which is rarely available in this setting.
Discussions

- Melioidosis exist in Cambodia and is likely under-diagnosed it thus may be an under-recognized cause of mortality and morbidity.

- Improved diagnostic microbiology capacity are needed in Cambodia.

- The identification of melioidosis in Cambodia has led to an increased awareness and understanding of this infection.
Dr. William Housworth
Director of AHC

Dr. Varun Kumar
Senior paediatrician

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