

# Emergence of Pediatric Meliodosis in Siem Reap, Cambodia

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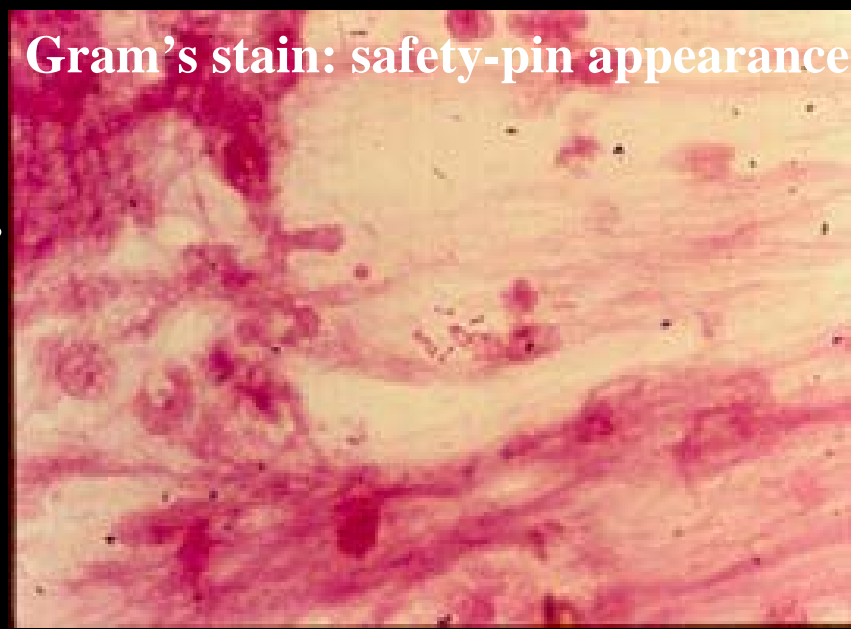


# Melioidosis

Gram's stain: safety-pin appearance

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



(Chaowagul W, *et al.*, 1989; Suputtamongkol *et al.*, 1994)

# 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)

# Cambodia



# Melioidosis in Cambodia

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## A first report of pulmonary melioidosis in Cambodia

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### Melioidosis is under-diagnosed in Cambodia:

- i) laboratories are not equipped to grow the bacterium
- ii) clinicians are not yet familiar with melioidosis

# Melioidosis in Cambodia

*B. pseudomallei* was isolated from 30% of soil samples

ranged from 1-5,000 (median 90 CFU/g, IQR 20-250CFU/g of soil)



## **Burkholderia pseudomallei** Antibodies in Children, Cambodia

Vanaporn Wuthiekanun,\* Ngoun Pheaktra,†  
Hor Putchhat,† Lina Sin,† Bun Sen,†  
Varun Kumar,† Sayan Langla,\*  
Sharon J. Peacock,\*‡ and Nicholas P. Day\*‡

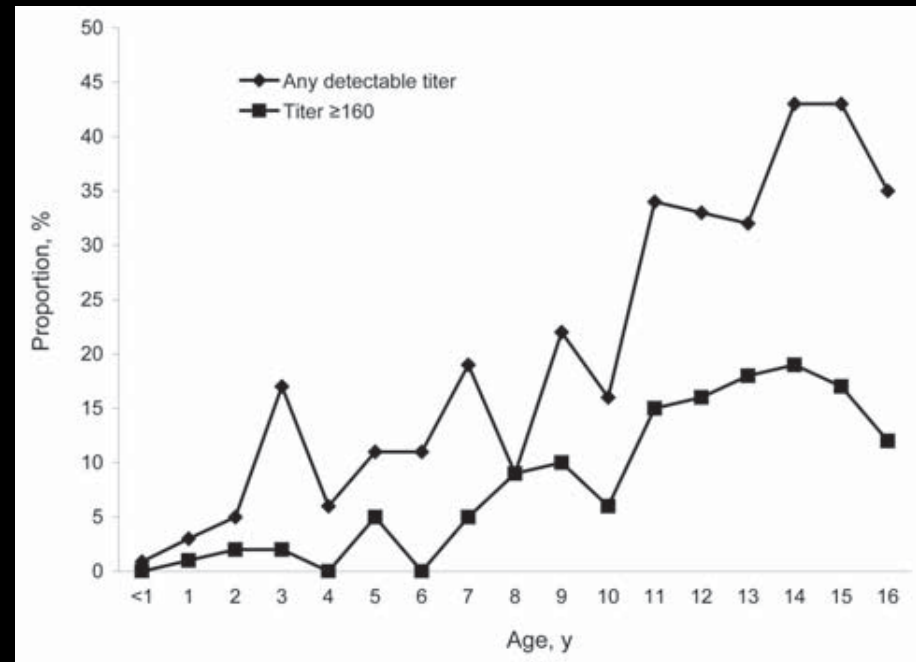
Antibodies to *Burkholderia pseudomallei* were detected in 16% of children in Siem Reap, Cambodia. This organism was isolated from 30% of rice paddies in the surrounding vicinity. Despite the lack of reported indigenous cases, melioidosis is likely to occur in Cambodia.

and to determine whether this organism could be isolated from their environment.

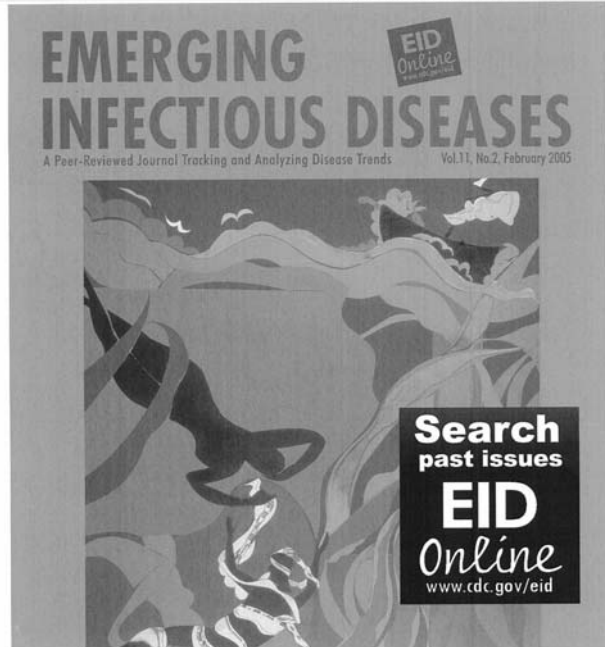
### The Study

A prospective, cross-sectional study was conducted at Angkor Hospital for Children, Siem Reap, from December 2005 through April 2006. Unselected consecutive serum samples were collected from children between birth and 16 years of age from the biochemistry and hematology laboratory of Angkor Hospital for Children, Siem Reap. Blood samples were collected from outpatients and inpatients. These blood tests were ordered by the primary physician for other reasons, and the sample used represented surplus material. Samples were centrifuged at 3,000 rpm for 10 min and the serum stored at -30°C. Target sample numbers were 40-60 per year group. An anonymous database was created to record sex, age, and indirect hemagglutination assay (IHA) titer. The presence and titer of antibodies to *B.*

Indirect hemagglutination assay titer for 968 children

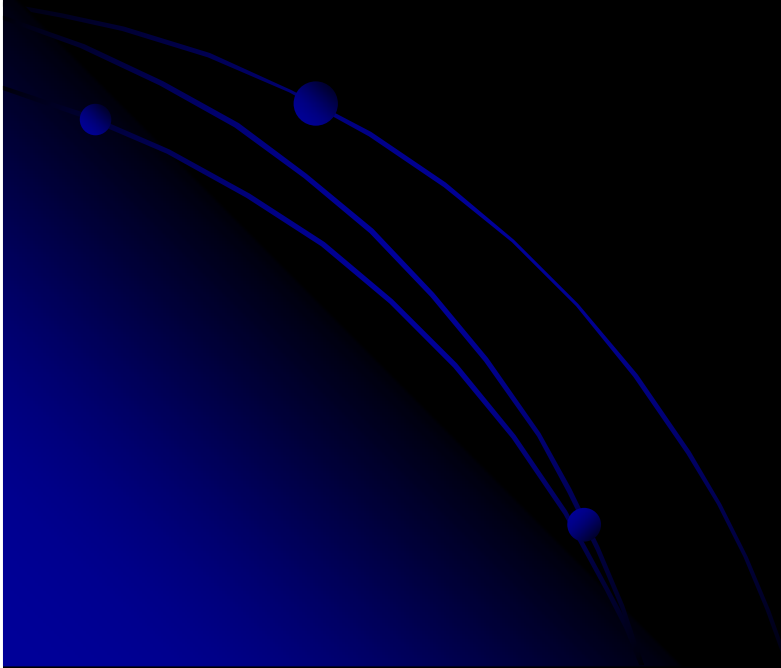


Antibodies to *Burkholderia pseudomallei* were detected in 16% of Children in Siem Reap



# **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.*



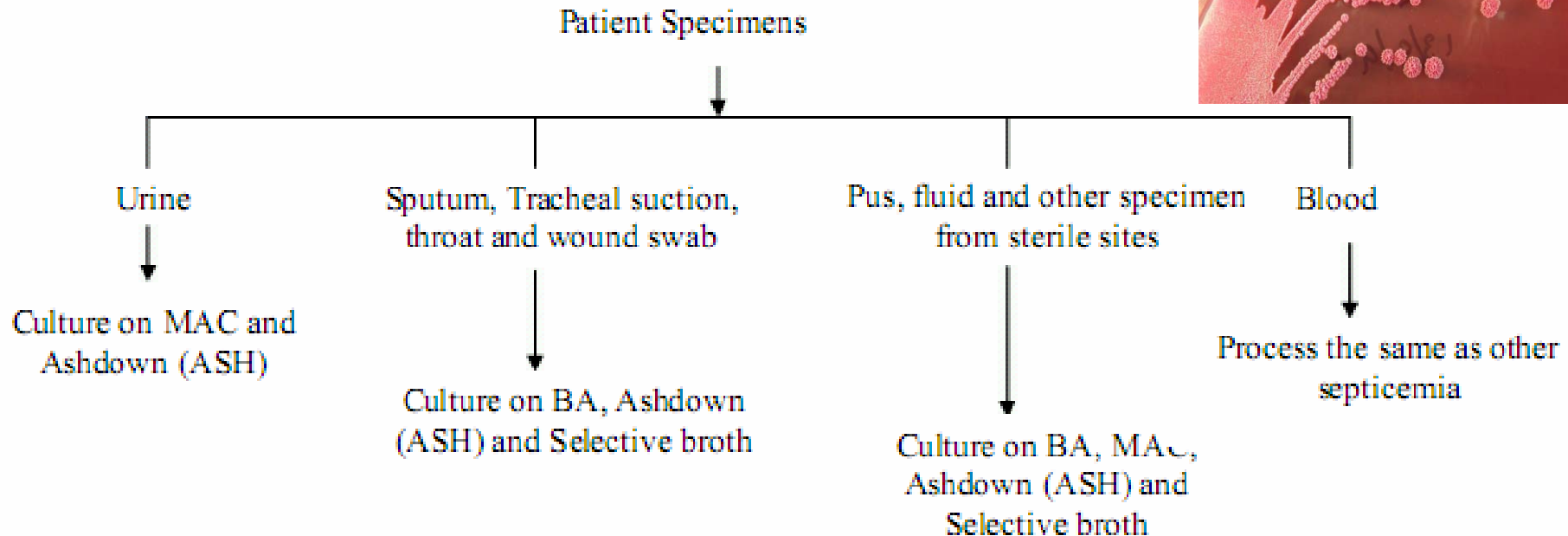


# Materials and Methods

- 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

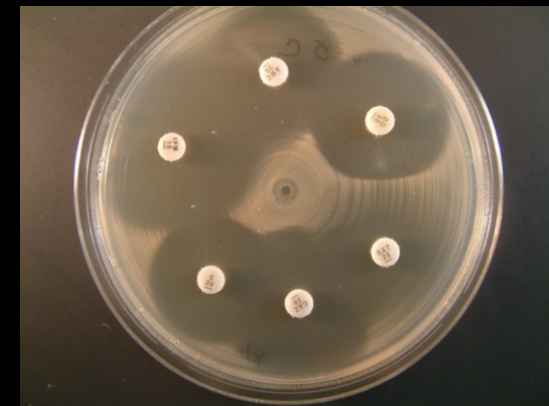
## Process for culture of *Burkholderia pseudomallei*



## Identifications

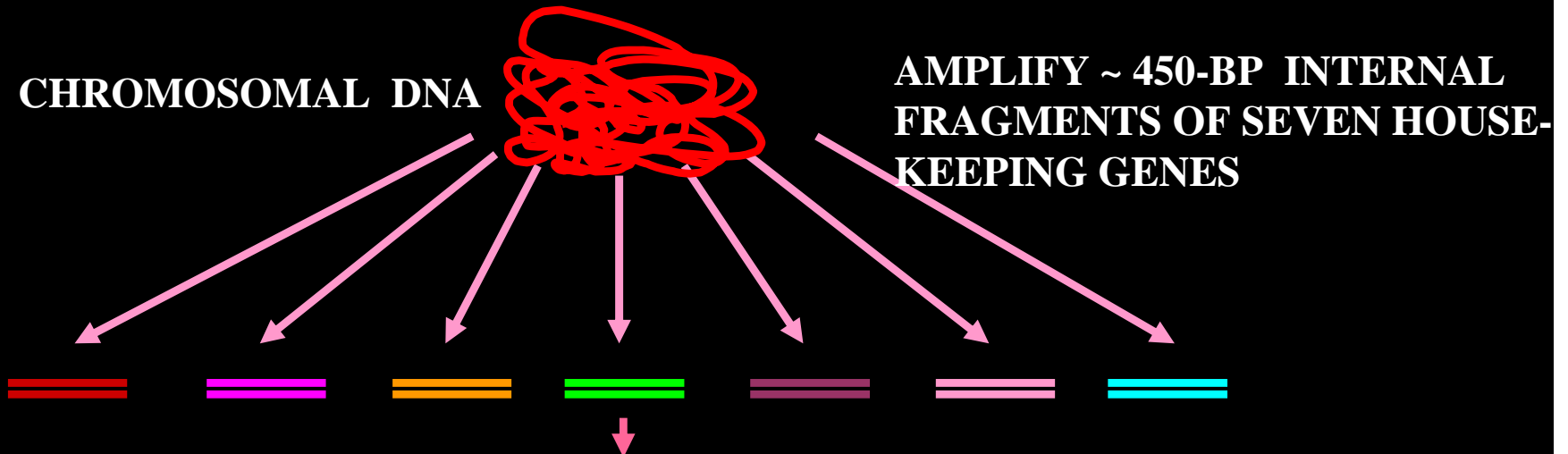
- Gram stain, oxidase test
- Colistin and gentamicin disc screening test
- Latex agglutination test
- API 20NE profile

## Susceptibility testing



# Bacterial genotyping

## Multi-Locus Sequence Typing (MLST)



**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](http://www.mlst.net))**

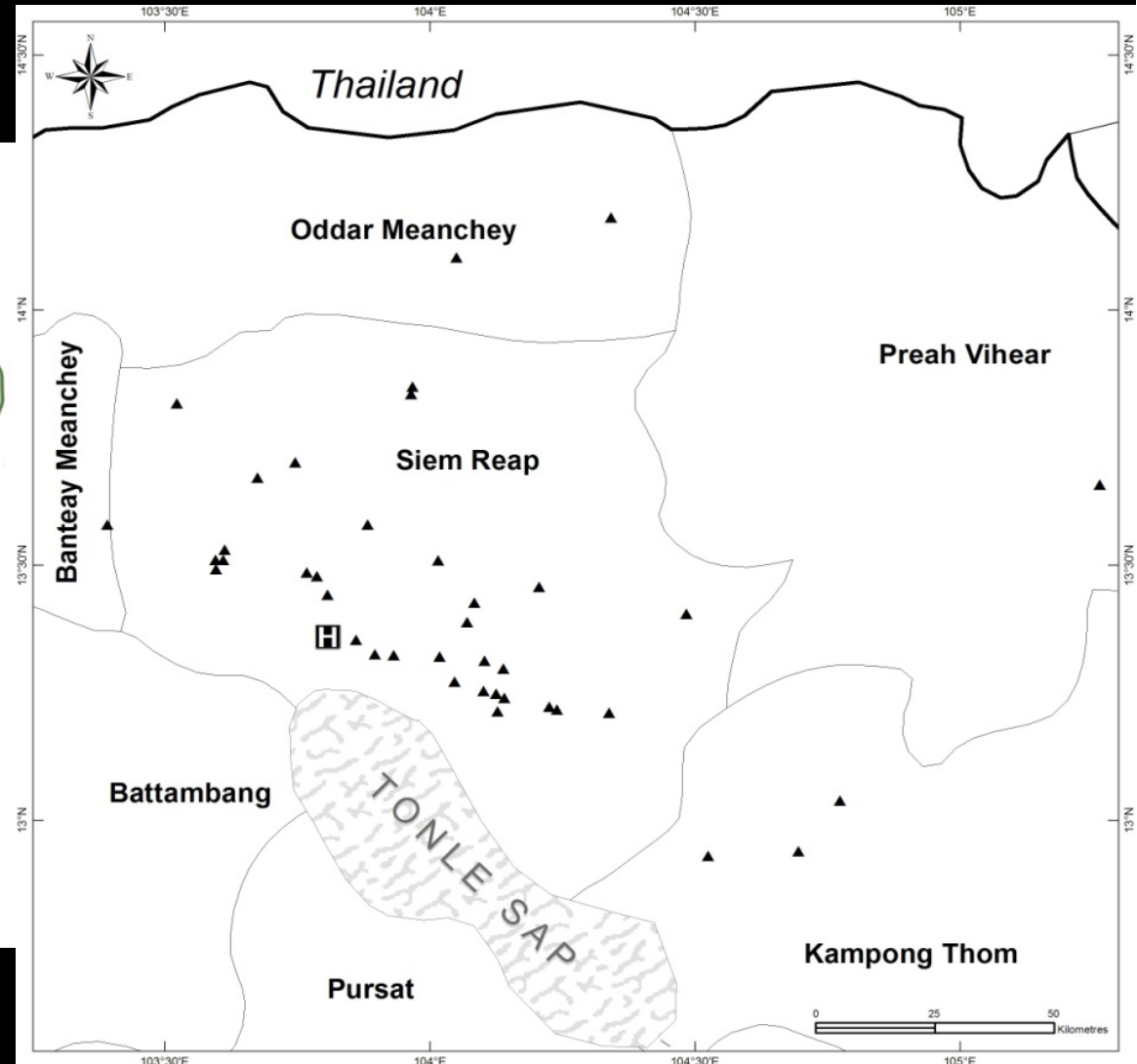
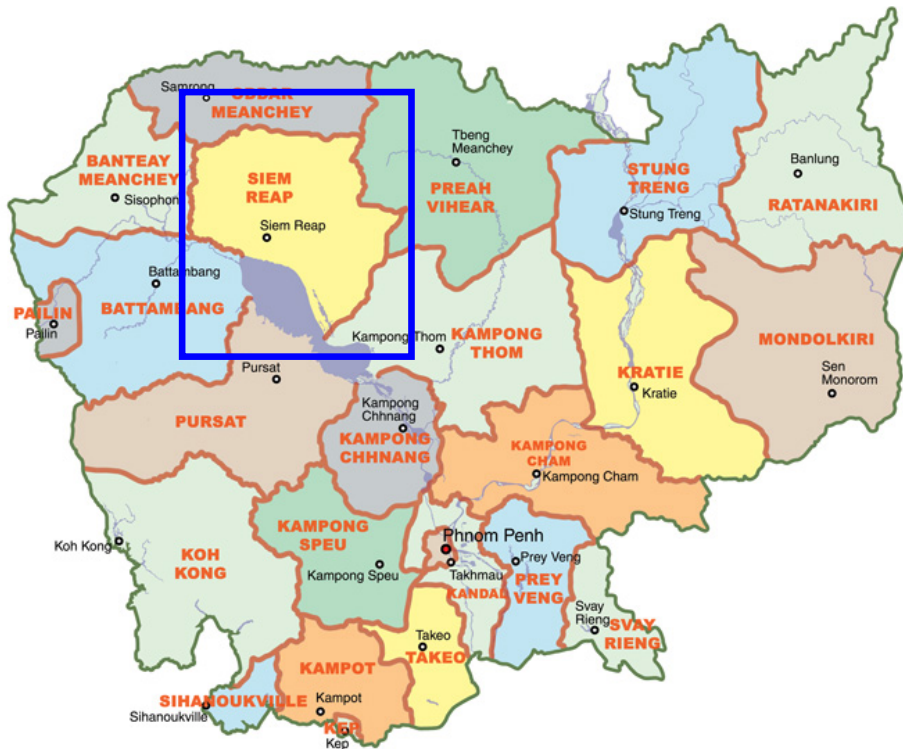
## Summary data for 39 children with melioidosis

# Results

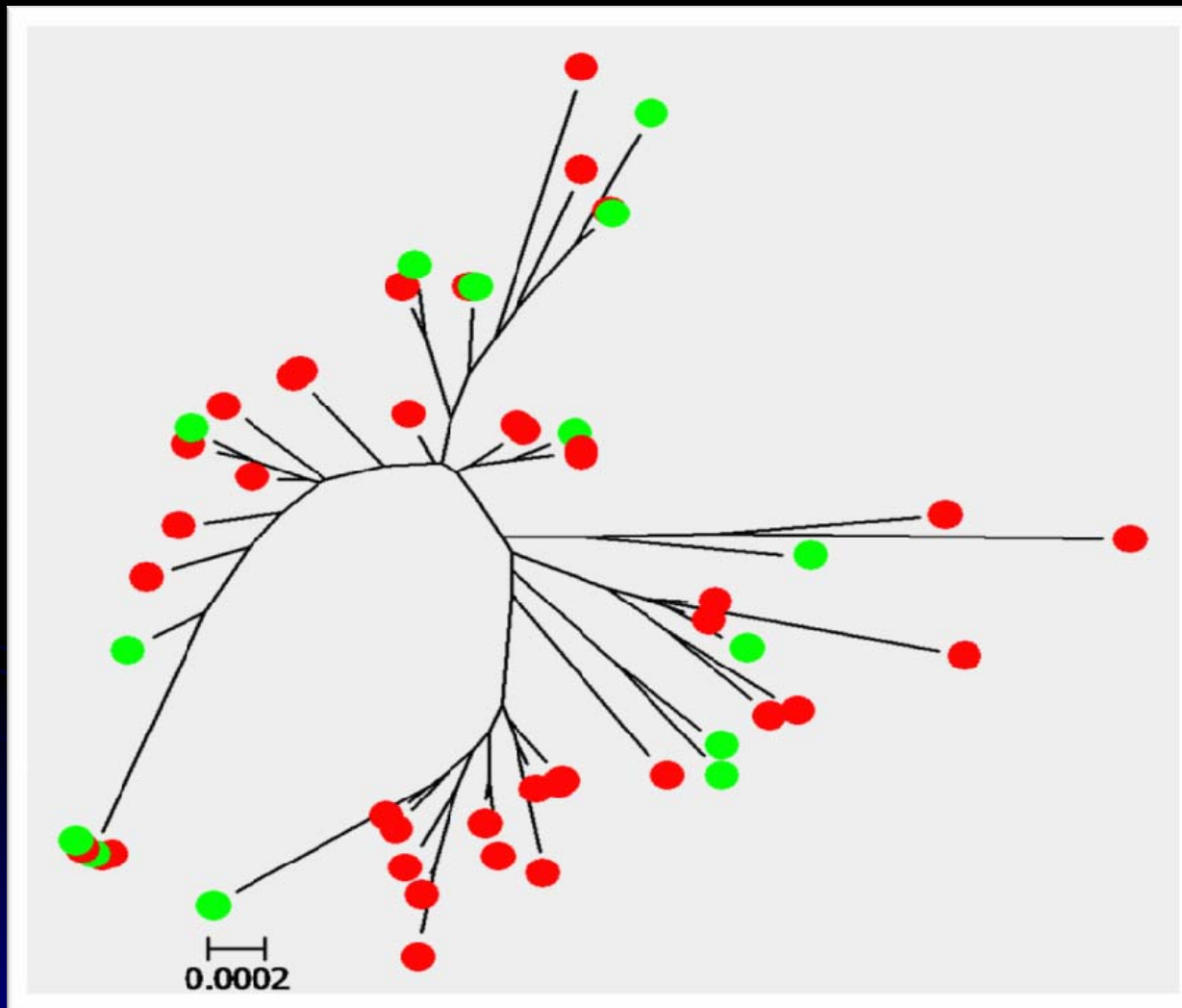
Variable	Number	Percentage
Male gender	15	38%
Age (yrs), median	<b>7.8</b>	
Ranges, yrs	1.6-16.2	
Interquartile range (IQR)	4.1-12.4	
<b>Source of isolate;</b>		
- Blood	9	23%
- Pus	<b>29</b>	74%
- Respiratory secretion	1	3%
<b>Severity of infection;</b>		
- Localized	27	69%
- Disseminated	12	31%
Died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

# 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

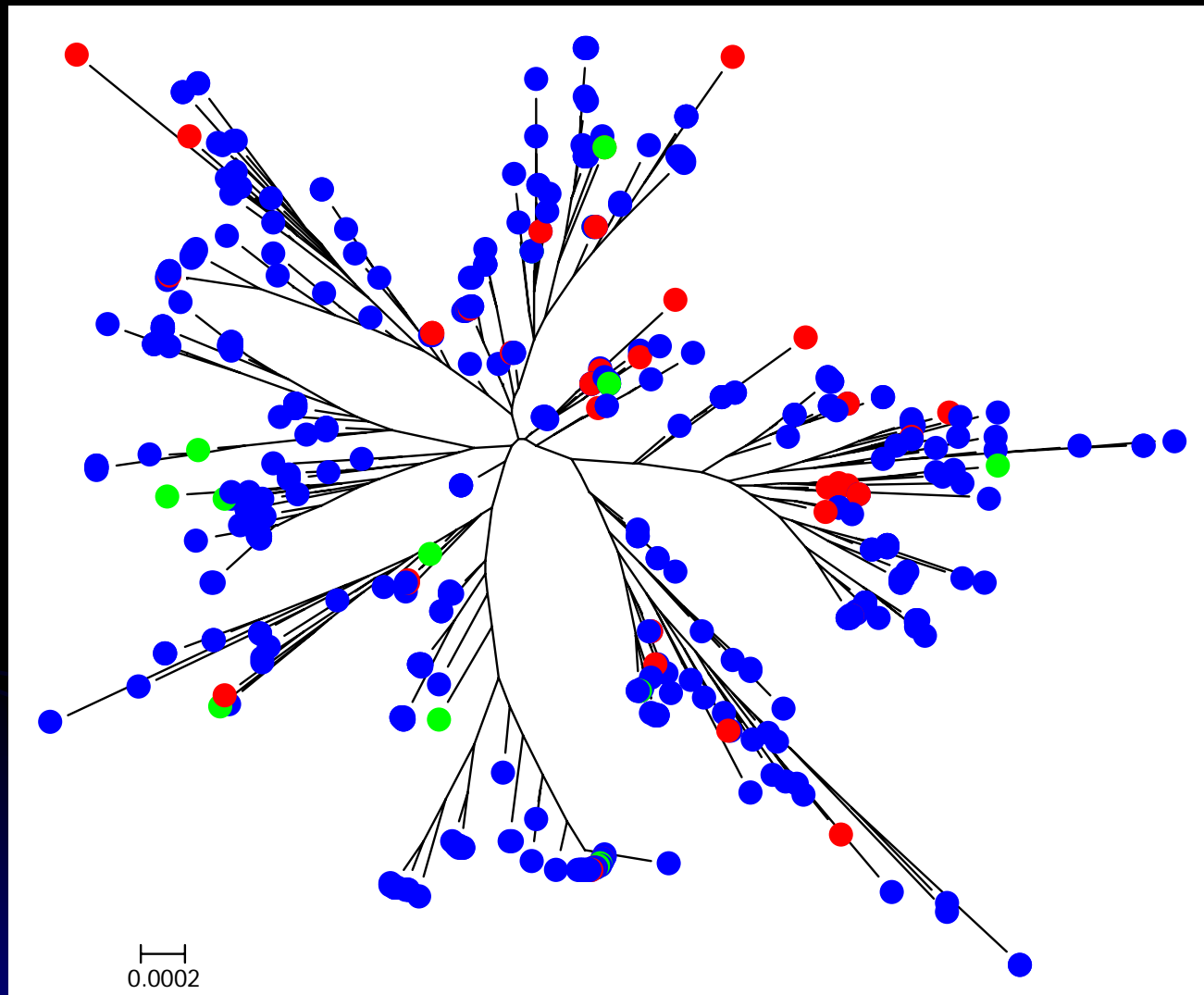


● Cambodia  
isolates, invasive  
(N=39)

● Cambodia  
isolates, soil  
(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



Cambodia isolate  
( ● red and ● green)  
(N=53)

● Thai isolates  
(N=462)

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](http://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.



**CHILDREN OF CAMBODIA**  
**ABOUT THE HOSPITAL.**  
**WAYS YOU CAN HELP.**  
**CONTACT US**

Every child has the right to a healthy and loving life. By opening your heart, **sharing your skill** or providing **support**, you can make a profound difference in the life of a child and the future of a nation.

**caring for our future**



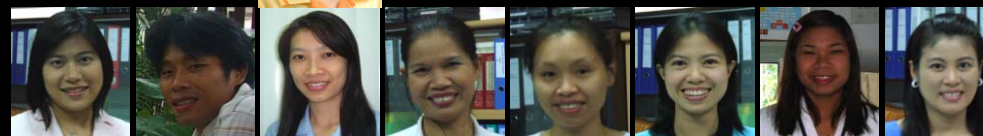
Angkor Hospital for Children, in Siem Reap, Cambodia, is a pediatric teaching hospital funded by the NGO **FRIENDS** OF THE ANGKOR



**Dr. William Housworth**  
 Director of AHC



**Dr. Varun Kumar**  
 Senior paediatrician



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