Molecular evidence of clusters of neonatal *Candida parapsilosis* candidaemia through sentinel laboratory-based surveillance, South Africa 2009-2010

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Why focus on neonatal candidaemia?

• Neonatal candidaemia is a common, deadly and costly hospital-associated disease

• Incidence of neonatal candidaemia is approximately 5 cases per 100,000 population

• Incidence of candidaemia due to *C. parapsilosis* is higher in neonatal vs. adult ICUs
Why focus on neonatal candidaemia?

• In a US-based study, cost was estimated to be $28,000 per patient

• Crude mortality is ~50%
Candida parapsilosis is an important invasive neonatal pathogen

- Most common pathogen after Candida albicans
- Comprises 3 groups (I, II, III) which cannot be differentiated using standard laboratory methods
  - C. parapsilosis (group I) is responsible for most cases
- Relatively low virulence but has the ability to adhere and form biofilms
Possible sources and routes of transmission

• Normal commensal of human skin
• Can be transmitted patient-to-patient on hands of health care workers with breaches in infection prevention and control practices
• Adheres tenaciously to foreign material and forms biofilms on patient-related hardware, e.g. intravenous catheters
• Proliferates rapidly in glucose- and amino acid-rich solutions
Important risk factors for neonatal candidaemia

• Low birth weight
• Compromised skin or mucosal integrity
• Long-term endotracheal intubation
• Mechanical ventilation
• IV catheters in-situ
Molecular typing of *C. parapsilosis*

- Molecular epidemiology can potentially show the persistence and transmission of related strains in hospital settings

- However, *C. parapsilosis* has two sets of chromosomes and few genetic differences are found among strains, even those that are unrelated
Older typing methods

- Several methods were used previously including MLST, electrophoretic karyotyping, RFLP and ITS sequencing
- Disadvantages
  - Limited ability to accurately determine genetic relatedness among strains
  - Non standardised methods
  - Poor inter-laboratory reproducibility
Microsatellite genotyping

- *C. parapsilosis* can be easily differentiated with high discriminatory power by microsatellite genotyping
  - Can detect micro-evolutionary variations
  - Facilitates detection of outbreaks
Hospital outbreaks of *C. parapsilosis* candidaemia

- Outbreaks of candidaemia have been described in neonatal ICUs (Romeo et al, 2013; Reiss et al, 2012)
  - Isolates from hands of health care workers have been found to be genetically similar to the NICU isolates (Sabino et al, 2015; Delfino et al, 2014)
  - Genotyping has been used to unmask “smouldering clusters” of candidaemia over years (Asmundsdottir et al, 2008)
Aim of this study

• To provide molecular evidence of clusters of hospital-associated *C. parapsilosis* fungaemia in South African neonatal units and thus provide further motivation for improved IPC practices
Methods

• Retrospective cross-sectional study design
  – Isolates submitted as part of TRAC-SA surveillance or from diagnostic laboratories for species-level identification
  – February 2009 through August 2010

• Case definition for this study: A patient aged ≤28 days admitted to any hospital with first isolation of *C. parapsilosis* from blood culture
Identification and genotyping of *Candida parapsilosis*

TRAC-SA surveillance, 2009-2010

Isolate/s on Dorset transport medium

Chromogenic agar medium

- Fluconazole MICs (BMD)
- VITEK 2 YST
  - If poor VITEK ID: API 20C AUX
    - If poor API 20C ID: API ID 32C
  - Molecular ID by sequencing of the ITS region (ribosomal gene)
    - Microsatellite genotyping
Microsatellite genotyping Methods

1. DNA extraction
2. PCR amplification of Microsatellite loci
3. Fragment analysis using genetic analyser
Methods

- A “cluster” was defined as a group of isolates that could be epidemiologically linked (by place and time) and contained three or fewer allele differences between them.

- The cluster analysis was restricted to 5 public-sector hospitals.
Results

• 2172 cases of candidemia were detected
• 1671 (77%) cases had viable isolates
• Of these, 393 cases (24%) occurred among neonates
  – 143 cases (37%) were caused by *C. parapsilosis*
  – 134 cases from 12 public-sector hospitals and 9 cases from private-sector hospitals
Results

• All isolates from these cases were confirmed to be *C. parapsilosis* by sequencing of the ITS region
18 of 79 genotypes were represented by ≥2 isolates

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of cases</th>
<th>Loci (bp)</th>
<th>Genotype frequency (%)</th>
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<td></td>
<td></td>
<td>B</td>
<td>G</td>
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<tr>
<td>Genotype 1</td>
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<tr>
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<td>126/126</td>
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<tr>
<td>Other genotype (1 cases each)</td>
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<td>NA</td>
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Relationship between genotype and cluster at 5 public-sector hospitals, n=117

<table>
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<tr>
<th>Cluster type</th>
<th>Number of genotypes</th>
<th>Number of isolates (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>14 (12)</td>
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<tr>
<td>2</td>
<td>17</td>
<td>37 (32)</td>
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<td>3</td>
<td>31</td>
<td>64 (55)</td>
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<td>Non-cluster</td>
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<td>2 (1)</td>
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<td>Total</td>
<td>59</td>
<td>117</td>
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Cluster type distribution at 5 hospitals, n=117

CHBA (n=73)
- Non-cluster
- Cluster 3
- Cluster 2
- Cluster 1

DGM (n=18)
- Non-cluster
- Cluster 3
- Cluster 2
- Cluster 1

TAD (n=8)
- Non-cluster
- Cluster 3
- Cluster 2
- Cluster 1

CMJA (n=3)
- Non-cluster
- Cluster 3
- Cluster 2
- Cluster 1

IAL (n=15)
- Non-cluster
- Cluster 3
- Cluster 2
- Cluster 1
Cluster distribution by month, Chris Hani Baragwanath Academic Hospital, n=73

2009

February March April May June July August September October November December

2010

January February March April May June July

Cluster 1
Cluster 2
Cluster 3
Non-cluster
Cluster distribution by month, Dr George Mukhari Hospital, n=18 (all cluster type 2)
Fluconazole susceptibility by hospital

CHB (n=73)  
DGM (n=18)  
TAD (n=8)  
CMJA (n=3)  
IAL (n=18)

- **Resistant**
- **Susceptible**
Cluster type vs. fluconazole susceptibility, Chris Hani Baragwanath Academic Hospital, n=73
Discussion

• With microsatellite genotyping, we demonstrated that genetically-related *C. parapsilosis* strains caused outbreaks in several public-sector hospitals

• Strains were introduced and then caused endemic disease in the same hospital
Discussion

• Cross-transmission among patients is most likely related to contaminated medical devices and surfaces and due to suboptimal IPC practices, including hand antisepsis

• However, this remains speculation because no IPC audits or environmental surveys were specifically conducted related to this study
Conclusion

• Using polymorphic microsatellite markers, we have shown that cases of *C. parapsilosis* fungaemia in public-sector hospital NICUs were caused by closely-related genotypes and there was molecular evidence of potentially-undetected outbreaks as well as intra-hospital and inter-hospital transmission.
Acknowledgements

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