Real-time polymerase chain reaction assays on dried blood spots for detection of Streptococcus pneumoniae in febrile children: a surveillance diagnostic method for resource-limited settings

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BACKGROUND

Streptococcus pneumoniae is the leading infectious cause of mortality in Nigeria. A major challenge in accurately determining the prevalence of pneumococcal disease is the lack of resources that make it possible to process diagnostic specimens. Dried blood spot (DBS) testing is an ideal method for diagnosis of infections in the developing world because of its low cost, minimal blood volumes involved, and ability for storage at ambient temperature.

METHODS

Children 5 years of age or under who presented to six hospital study sites throughout northern and central Nigeria with an acute febrile illness (Temp >38.5 °C) associated with difficulty breathing or altered consciousness, were prospectively enrolled from September 2011 through June 2015. Blood was obtained for culture using the automated Bactec™ incubator system and also spotted onto Whatman 903 and/or Whatman FTA filter paper. DBS DNA extractions were used as a template in real-time-PCR assays with the lytA gene, and RNase P was used as a control to assess the extraction of human cellular DNA.

RESULTS

A total of 357 DBS evaluated by PCR• Complete data was available for 261 (73.1%; Table 1). Of 357 DBS:• 8 were culture-positive (2.2%).• 9 were PCR-positive (2.5%).• One culture-confirmed case was missed. • PCR identified one culture-negative specimen from a high-risk group, as well as in a normal healthy control (Table 2).

HYPOTHESIS

Using DBS collected from a prospective cohort of febrile children in Nigeria, we hypothesize that molecular methods would be more sensitive than culture in the detection of S. pneumoniae bacteremia.

Table 1. Characteristics of febrile patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=261 (%)</th>
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<tbody>
<tr>
<td>Median age, months (range)</td>
<td>14 (0-132)</td>
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<tr>
<td>&lt;2 months</td>
<td>57 (21.8)</td>
</tr>
<tr>
<td>2~12 months</td>
<td>54 (20.7)</td>
</tr>
<tr>
<td>12~60 months</td>
<td>128 (49.0)</td>
</tr>
<tr>
<td>&gt;60 months+</td>
<td>22 (8.4)</td>
</tr>
<tr>
<td>Male sex</td>
<td>139 (53.3)</td>
</tr>
</tbody>
</table>

Blood culture

No growth 169 (64.8) Contaminants* 44 (16.9)
Salmonella Typhi 15 (5.7)
Streptococcus pneumoniae 8 (3.1)
Salmonella spp 7 (2.5)
Staphylococcus aureus 3 (1.1)
Escherichia coli 3 (1.1)
Candida spp 2 (0.8)
Chryscosomona luteola 2 (0.8)
Miscellaneous*** 8 (3.1)
Diagnosis at admission

Malaria 83 (31.8)
Sepsis 78 (29.9)
Respiratory tract illness/pneumonia 63 (24.1)
Diarrhea/dysentery/enteritis 10 (3.8)
Tonsillitis/pharyngitis 7 (2.7)
Enteric fever 5 (1.9)
Skin infection 4 (1.5)
Meningitis 4 (1.5)
Cardiac disease 3 (1.1)
Miscellaneous** 5 (1.9)

*Contaminants: alpha-hemolytic strep, Bacillus spp, coagulase-negative staph, Micrococcus spp., non-hemolytic strep, Pantoea
**Citrobacter spp., Enterobacter spp., Enterococcus spp., Haemophilus spp., Klebsiella spp., Pseudomonas spp., Salmonella Paratyphi
***Dehydration, Neonatal tetanus, Osteomyelitis, Sickle cell crisis, Viral

Table 2. Detection of Streptococcus pneumoniae on clinical specimens

<table>
<thead>
<tr>
<th>S. pneumoniae</th>
<th>S. pneumoniae</th>
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<tbody>
<tr>
<td>culture-positive</td>
<td>culture-negative</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>S. pneumoniae PCR-positive</td>
<td>S. pneumoniae PCR-negative</td>
</tr>
<tr>
<td>252</td>
<td></td>
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</tbody>
</table>

CONCLUSION

Real-time PCR detection of S. pneumoniae in DBS led to an equivalent detection of the pathogen compared to conventional blood culture, and was able to detect S. pneumoniae in culture-negative specimens. However, S. pneumoniae was also detected in a normal healthy control, which deserves further study. PCR assays from DBS are a promising diagnostic method suitable for conducting surveillance and can provide precise assessments of the effect of conjugate vaccines in resource-limited settings.

Acknowledgements

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References

3. Tarrago D, Fernell A, Sanchez-Tata T, Antonio JA, Munoz-Almagro C, Esteva G, Dobbins and the Minnesota Department of Health for providing pneumococcal isolates and technical guidance. We thank GE Healthcare Life Sciences for donating Whatman FTA cards and reagents for this study.