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EVALUATION OF DRIED BLOOD SPOTS AMONG CHILDREN <5 YEARS OLD FOR *STREPTOCOCCUS PNEUMONIAE* DETECTION AND SEROTYPING IN RURAL MOZAMBIQUE

F. C. Pimenta[1*], B. Moiane[2], A-K. L. Venero[1], I. Moura[1], S. Larson[1], S. Massora[2], A. Chaúque[2], H. Mucavele[2], B. Sigaúque[2], F. C. Lessa[1], C. Whitney[1] and M. G. Carvalho[1].

Author affiliations: [1]Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [2]Centro de Investigação em Saúde de Manhiça, Acute Respiratory & Invasive Bacterial Infections Research Unit, Maputo, Mozambique.

BACKGROUND

- ***S. pneumoniae*** **is the leading cause of bacterial pneumonia and meningitis, yet collection of invasive specimens and diagnosis by culture or PCR remain challenging.**
- **Dried blood spots (DBS) have been used for viral and parasitic diseases detection because of their low cost, minimal blood volumes involved, and room temperature storage viability. However, little is known about their use as a diagnostic for bacterial infections.**

OBJECTIVE

- **We evaluated the use of DBS for pneumococcal and *Haemophilus influenzae* detection in children under 5 years old.**

METHODS

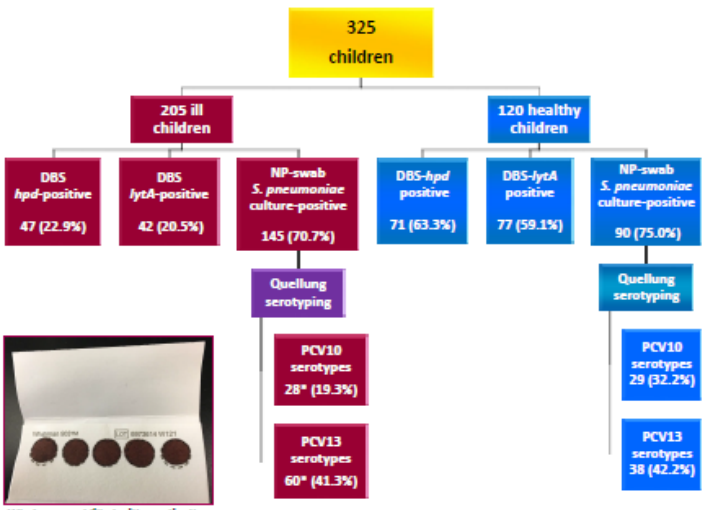
- **DBS and nasopharyngeal (NP) swabs were collected from ill children enrolled through pneumonia surveillance and from healthy children in Manhiça District, a rural area in Southern Mozambique.**
- **Blood is routinely collected for culture at admission to Manhiça District hospital for all children <2 years of age and for children 2-14 years of age with axillary temperature ≥ 38°C. For DBS, blood was collected through finger prick and spotted onto Whatman 903 filter paper cards.**
- **NP swabs were collected in STGG media and 200ul were used for pneumococcus isolation and identification using optochin and bile solubility tests[1]. *S. pneumoniae* isolates were serotyped by Quellung reaction.**
- **200uL of STGG from all culture negative NP swabs were submitted to DNA extraction using the MagNA Pure Compact instrument.**
- **The five DBS from each card were treated with Buffer ATL, proteinase K (from Qiagen) and buffer #4 (MagNA Pure LC DNA – Isolation Kit III) before nucleic acid purification by MagNA Pure Compact instrument.**
- **Quantitative PCR (qPCR) looked for:**
  - ***lytA*** **(for detection of *S. pneumoniae*)[1]**
  - ***hpd*** **(for detection of *H. influenzae*)[2]**
  - **Specific capsule genes (mostly *wzy*) for serotyping[3]**
- **DNA extracts were stored at -20C until qPCR was performed using Quanta Biosciences PerfeCTa® qPCR ToughMix®, Low ROX™.**

REFERENCES

1. Da Gloria Carvalho *et al.* 2010. J. Clin. Microbiol. 48: 1611-1618
2. Wang et al. 2011. Int J Med Microbiol. 301:303-309
3. Pimenta *et al.* 2013. J Clin Microbiol. 51(2):647-652

RESULTS

Figure 1: DBS and NP swabs results



- **Pneumococci were concurrently identified in the DBS and NP swabs of 67/120 (55.8%) healthy children, of which 11 shared the same serotype, and 36/205 (17.6%) ill children, of which 10 shared the same serotype.**
- **Where results from corresponding DBS and NP-swabs could be compared, we detected the same serotype/serogroup for 10/13 (76.9%) ill and 11/24 (45.8%) healthy children.**
- **Blood culture results were available for 185/205 (90.2%) ill children; *S. pneumoniae* was isolated from 6 (3.2%). Pneumococci were concurrently identified in the DBS and NP swabs of all 6 children, and in three cases the same serotype was identified in the blood, DBS, and NP swab, and were different in the others. *H. influenzae* b was isolate in the blood culture and concurrently detected in the DBS.**

CONCLUSIONS

- **We found more pneumococcal and *H. influenzae* DNA in healthy children's DBS than in DBS from ill children, suggesting that DBS testing did not distinguish colonization from disease.**

CONTACT INFO

Fabiana C Pimenta
Microbiologist
Respiratory Diseases Branch
U.S. Centers for Disease Control and Prevention
gzy7@cdc.gov

Maria da Glória Carvalho
Microbiologist
Respiratory Diseases Branch
U.S. Centers for Disease Control and Prevention
msc8@cdc.gov

cism centro de investigação em saúde de manhiça