Carriage rates, circulating serotypes and antibiotic resistance among *Streptococcus pneumoniae* in healthy infants in Yei, South Sudan

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Summary

The carriage of *Streptococcus pneumoniae,* serotypes, antimicrobial susceptibility patterns and disease development are poorly understood in Yei. Availability of affordable antibiotics over the counter, lack of laboratory infrastructure and high rates of penicillin resistance have the potential to aggravate rates of childhood mortality associated with *Streptococcus pneumoniae*. There is an urgent need to strengthen microbiological and public health services.

Introduction

The burden of lower respiratory tract infections is significantly greater in the developing world compared with developed countries (1). *Streptococcus pneumoniae* is responsible for 70-80% of severe pneumonia cases in the African continent (2). It is frequently associated with otitis media, bacteraemia and meningitis. The incidence of pneumococcal infections is greater in high-risk groups especially infants. Environmental factors such as crowding and air pollution also contribute to the risk (3). In tropical regions, invasive pneumococcal diseases (IPD) occur frequently during the cold, dry months (4). However, in temperate climates, IPD are most often observed during the winter months (5). The mucosal epithelium of the nasopharynx is the primary site of pneumococcal colonization with an average duration of colonization between 2.5 and 4.5 months (6).

Capsular serotypes causing nasopharyngeal colonization and infections, as well as the development of antibiotic resistance, vary according to age, geographic location, and socioeconomic status (7, 8). However, there is an apparent link between penicillin resistance and high level antibiotic consumption (9). There is a substantial overlap between the serotypes that are carried and those that are recovered from IPD (6, 10). Some pneumococcal serotypes colonizing the nasopharynx have little tendency to cause IPD (11).

Pneumococcal carriage rates and patterns of antibiotic-resistance are important in determining response to antibiotics or vaccines, especially when these may be effective against only a subset of strains or serotypes. This report describes isolation, carriage rates and antibiotic-resistance in pneumococci among healthy infants in Yei, South Sudan. The report also highlights essential issues for improving diagnostics, public health services and other infrastructures.

Methodology

Population, location and samples

Nasopharyngeal swabs were taken from 38 healthy infants aged between 2 weeks and 12 months, during a single vaccination session at Martha Clinic. A verbal explanation of the purpose of the study was given to and consent obtained from the parents of the children prior to sampling. Those who agreed to participate were questioned regarding the age of the child, recent antibiotic therapy and any hospitalization in the past two weeks. A flexible per-nasal twisted wire with rayon tip (Sterilin®: Newport, NP11 3EF, UK) was used to take the swabs and transported in Sterilin Transport Swabs with Charcoal/ Aimes Media. The lack of incubators, -80°C freezers and regular electricity made storage and transport of samples suboptimal.

Culture, bacteriological identification, antibiotic sensitivities and capsular serotyping

Forty-eight hours after taking the swabs, they were cultured on Colombia blood agar plates plus Optochin disks. Plates were incubated for 24-48 hours at 37°C with

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Table 1. Results of antibiotic sensitivities and circulating serotype

Infant No.	Age in months	Serotype	Penicillin* (MIC)	Cotrimox-azole	Erythro-mycin	Tetracy-cline	Cefachlor	Chloram-phenicol	Linezolid
1	9	- **	R (0.5)	R	S	S	S	S	S
4	9	23F	R (0.25)	R	S	S	S	S	S
8	9	-	R (1)	R	S	R	S	S	S
11	2	15A/ 15F	R (0.25)	R	S	S	S	S	S
12	1	6A/ 6B	R (0.5)	R	S	S	S	S	S
20	9	23F	R (0.5)	R	S	S	S	S	S
21	1	7C/ 7B	R (0.5)	R	S	S	S	S	S
23	2	-	R (0.12)	R	S	S	S	S	S
27	1.5	23A	R (0.25)	R	S	S	S	S	S
30	2.5	6A/ 6B	R (0.5)	R	S	R	S	S	S
31	12	19A	R (0.25)	R	S	R	S	S	S
34	3	6A/ 6B	R (0.5)	R	S	R	S	S	S
36	5	6A/ 6B	R (0.25)	R	S	S	S	S	S
37	2	19F	R (0.5)	R	S	S	S	S	S
Colonization Rate		39 %	100 % Resistance Rate	100 % Resistance Rate	0 % Resistance Rate	25 % Resistance Rate	0 % Resistance Rate	0 % Resistance Rate	0 % Resistance Rate

**All isolates expressed low level penicillin resistance; the values represent minimum inhibitory concentration (MIC) for each isolate*

***- Isolates not been serotyped by our PCR. R = Resistant, S = sensitive*

5% CO2 in the microbiology department at the Royal Hampshire County Hospital, Winchester, UK. Growth of pneumococci was identified from colony morphology and optochin sensitivity. Sensitivity to oxacillin (1μg), chloramphenicol (10μg), erythromycin (5μg), cefaclor (30μg), linezolid (10μg), cotrimoxazole (25μg) and tetracycline (30μg) (MAST, Liverpool, UK) was tested using the BSAC method (12) on ISO with 5% horse blood (E&O, Scotland, UK). Penicillin MICs of the oxacillin resistant serotypes were tested by using penicillin E-test (Oxoid, Basingstoke, UK)) on the same media according to the British Society of Antimicrobial Chemotherapy method (12). Pneumococcal isolates were transported to Sir Henry Welcome Laboratories, University of Southampton School of Medicine, Southampton, UK and stored on cryobeads (Microbank, Pro-Lab Diagnostics, Wirral, UK) at -80oC.

In order to prepare genomic DNA for capsular polymerase chain reaction (PCR) one bead was removed from the storage vial and streaked onto a Columbia blood agar plate plus optochin disc. Single colonies were suspended in 200l lysis buffer (10mM Tris, 100mM EDTA, 0.5% (weight/volume SDS) and incubated at 37oC for 1 hour. Genomic DNA (gDNA) was prepared from this lysate using the QiaAmp DNA kit (Qiagen, UK). Pneumococcal capsular typing was performed on genomic DNA isolated from sub-cultured isolates by multiplex-PCR following the method described by the Centers for Disease Control (13, 14).

Results

Every parent who was approached to participate in the study agreed to do so. None of the infants had been vaccinated with pneumococcal vaccines. No parent reported the use of antibiotics for their child and no child had been hospitalized in the 14 days prior to sampling. Fourteen (39%) of the 38 infants carried *S. pneumoniae* in the nasopharyngeal swabs.

Circulating serotypes and antibiotic sensitivities are shown in (Table 1). Our method was not able to serotype three isolates (infants 1, 8 and 23) as these isolates tested positive for the internal control for a universal

pneumococcal capsular gene (*cps*A) suggesting that a capsular locus is present in these strains. Based on these findings, 50% of the serotypes we identified in this group are covered by the seven valent pneumococcal vaccines which contains serotypes [4, 6B (potentially cross cover 6A), 9V, 14, 18G, 19F, 23F]. The ten and thirteen valent vaccine types would not provide additional coverage.

Discussion

Epidemics of pneumococcal disease have been reported in many communities before the discovery of antimicrobial agents and in other situations where people lived in crowded conditions(6). With better living conditions and the accessibility to antimicrobial agents, outbreaks of this disease have been infrequent. Carriage rates of 71.9% and 85% have been reported in infants from Zambia and Botswana respectively (15).

In this study we discovered 39% nasopharyngeal carriage among infants in Yei. Obtaining samples was not problematic. Lack of laboratory facilities (working incubators, culture media and regular electricity) meant that the conditions in which samples were kept and transported were not ideal. Availability of a better equipped laboratory would probably have increased recovery rates. Commonly isolated serotypes in this study included 6A/B, 19A, 19F and 23F. These serotypes are identified globally as common causes of IPD among children. None of the infants in our study was colonized with serotype 14 which is the most commonly identified serotype worldwide in children under aged five years (16). Children in Yei receive their medical care through the facilities of Yei Civil Hospital, Martha clinic and other local clinics. Local shops and pharmacies sell antibiotics without prior prescription. There is no antibiotic advice or protocol for use in these clinics and the hospital for otitis media, bronchitis, pharyngitis and lower respiratory infections. All the isolates proved to be resistant to cotrimoxazole and intermediately resistant to penicillin. Various factors may have contributed to this finding including over prescription, availability of affordable antibiotics without prescription, frequent use and multiple exposures to the same antibiotics, empirical therapy from prescribers and non compliance by patients leading to selection of more resistant strains.

Even though the sample size was small, our study highlights the increasing problem of antimicrobial resistance particularly among *S. pneumoniae.* This in turn complicates therapy of such infections. Penicillin seems to be no longer useful for the treatment of complicated infections due to this organism in this area. Although other agents like erythromycin, tetracycline, chloramphenicol and third generation cephalosporins can be used, clinicians and prescribers must know that the patterns of resistance continue to change and therefore constant epidemiological surveillance is needed for judicious antibiotic prescribing.

Figure 1. Microbiology laboratory at Yei Civil Hospital

Conclusion

The extents of pneumococcal carriage and disease, serotypes, and antimicrobial susceptibility patterns have been poorly described in South Sudan. The lack of quality diagnostic services with high rates of antimicrobial resistance may have the potential to seriously exacerbate childhood mortality associated with *S. pneumoniae.* A comprehensive, extensive-scale surveillance of both clinical and community isolates is necessary to identify serotypes and the extent of drug-resistant strains of *S. pneumoniae* in South Sudan, to allow management and prevention strategies to be established before options become limited. Therefore the priorities recommended to the government of South Sudan are:

- Urgent measures to strengthen microbiology laboratory and public health services (see Figure 1).
- Exploration of ways of addressing the lack of basic infrastructure.
- Continued monitoring of antimicrobial resistance.
- Continued professional education of all within the healthcare system including private pharmacies.

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Ethical approval was granted for this study by the South

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Transparency declaration

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References

1. Murray CJL, Lopez AD. Global mortality, disability, and contribution of risk factors. Global Burden of Disease Study. *Lancet*. 1997; 349: 1436–1442.
2. Mthwalo M, Wasas A, Huebner R, et al. Antibiotic resistance of nasopharyngeal isolates of Streptococcus pneumoniae from children in Lesotho. Bulletin of the World Health Organisation. 1998; 76: 641-650.
3. Heubner R, Wasas A, Klugman K. Prevalence of nasopharyngeal antibiotic resistant pneumococcal carriage in children attending private paediatric practice in Johannesburg. *South African Medical Journal*. 2000; 90: 1116-1121.
4. Gordon S, Walsh A, Chaponda M. Bacterial meningitis in Malawian adults: Pneumococcal disease is common, severe and seasonal. *Clinical Infectious Diseases*. 2000; 31: 53-57.
5. Saeed K, Dryden M, Paget C, et al. Invasive pneumococcal disease: epidemiological features and vaccine coverage. *Vaccine in practice*. 2010; 3 (2): 1-4.
6. Gray BM, Converse GM, Dillon HC. Epidemiologic studies of Streptococcus pneumoniae in infants: Acquisition, carriage, and infection during the first 24 months of life. *J Infect Dis*. 1980; 142: 923-933.
7. Block SL, Harrison CJ, Hedrick JA, et al. Penicillin-resistant Streptococcus pneumoniae in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. *Pediatric Infectious Disease Journal*.1995; 14: 751–9.
8. Harrison JA, Hedrick RD, Tyler RA, et al. Chartran. Penicillin-resistant Streptococcus pneumoniae in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. *Pediatrics Infectious Diseases Journal*. 1995; 14: 751-759.
9. Reinert RR, Queck A, Kaufhold A, et al. Antimicrobial resistance and type distribution of Streptococcus pneumoniae isolates causing systemic infections in Germany. *Clin. Inf. Dis*. 1995; 21: 1938-1401
10. Inostroza J, Trucco O, Prado V, et al. Capsular serotype and antibiotic resistance of Streptococcus pneumoniae isolated in two Chilean cities. *Clinical Diagnosis Laboratory Immunology*. 1998; 5: 176-180.
11. Brueggemann AB, Griffiths DT, Meats E, et al. Clonal relationships between invasive and carriage Streptococcus pneumoniae and serotype- and clone-specific differences in invasive disease potential. *J Infect Dis*. 2003; 187: 1424–1432
12. BSAC Standardized Disc Susceptibility Method Version 9.1 March 2010. available at : http://www.bsac.org.uk/Susceptibility+Testing/Breakpoints
13. Pai R, Gertz RE, Beall B. Sequential Multiplex PCR Approach for Determining Capsular Serotypes of Streptococcus pneumoniae Isolates. *J Clin Microbiol* 2006 Jan; 44(1): 124-31.
14. CDC. PCR Deduction of Pneumococcal Serotypes. 2010 03.03. [cited 2010 03.06.]; Available from: www.cdc.gov/ncidod/biotech/strep/pcr.htm .
15. Graneord J (2001). A community- based study of carriage and antibiotic resistance of streptococcus pneumoniae in children aged 5 and under in a Northern Tanzanian region. Unpublished data.
16. Pneumococcal Global Serotype Project (GSP) Summary Report (Stage 1; Version 1) for SAGE meeting November 6-8, 2007 October 18, 2007 Version