

CARRIER STATE OF *Streptococcus pneumoniae* IN THE NASOPHARYNX OF SICKLE CELL CHILDREN IN MULAGO HOSPITAL - KAMPALA, UGANDA.

¹Augustine, KM. Ssevviri, ¹Henry Kajumbula and ^{1*}Deogratias, H. Kaddu-Mulindwa

¹Department of Medical Microbiology & Molecular Biology, College of Health Sciences, School of Biomedical Sciences, Makerere University, P.O. Box 7072 Kampala, Uganda.

ABSTRACT.

Introduction: Invasive pneumococcal disease has been recognised as a serious complication of homozygous sickle cell (SS) disease since the first description of this association (Wollstein & Kriedel, 1928). In Uganda there was also a dearth of *Streptococcus pneumoniae* as a cause of septicemia (Monica Etima, 2002, personal communication, Nantanda, 2006, Katureebe, unpublished data 2007). No studies in sickle cell children have been done before in Uganda.

Objectives: The objective of this present study was to address the rate of nasopharyngeal carriage of *Streptococcus pneumoniae* in SS children attending the Sickle Cell Clinic in Kampala, Uganda and the antibiotic sensitivity of the isolated organisms.

Design: A descriptive cross-sectional study.

Setting: The study site was the Sickle Cell Clinic of Mulago Hospital and Complex.

Methods: The patients attended the Sickle Cell Clinic of Mulago University Teaching Hospital and the study was restricted to clinically well subjects with SS aged less than 6 years. Nasopharyngeal samples were taken for culture in all and the parents /guardians were questioned on the use of antibiotics over the 4 preceding weeks and for the history of pneumococcal immunization.

Results: *S. pneumoniae* was found in 27 (33%) of the study group (Table 1), occurring in 2/23 (9%) of those with recent hospitalization and in 25/58 (43%) of those without ($\chi^2 = 8.8$, $p=0.003$) where the p-value was obtained with Fisher's Exact test. Two subjects had received pneumococcal immunization and neither carried the organism. Antibiotic usage, usually for persistent coughs, was reported in 64/81 (79%) subjects (Table 2). Antibiotic sensitivity of isolated organism showed all to be penicillin resistant but sensitive to other commonly used antibiotics.

Conclusion: The nasopharyngeal carrier rate of *S. pneumoniae* in SS children aged below 6 years is high in Uganda and penicillin resistance is high among the isolated organisms. Further to that study on the prevalence or incidence of SCD in children in Uganda is recommended as this information is missing.

INTRODUCTION

Invasive pneumococcal disease has been recognized as a serious complication of homozygous sickle cell (SS) disease since the first description of this association by Wollstein and Kriedel, (1928). Since then there has been ample evidence that patients are more prone to pneumococcal meningitis (Robinson and Watson 1966, Barret-Connor 1971) and to pneumococcal septicaemia (Lobel and Bove 1982, Powars et al 1995). Indeed, the above studies indicate that until effective prophylaxis was introduced in the mid 1980's, *S. pneumoniae* accounted for 70 - 90% of septicaemic illness in SS disease in the US and the Caribbean. These observations contrast with those from Equatorial Africa where *S. pneumoniae* is an uncommon cause of septicaemic illness in SS disease. In, for example, the Democratic Republic of Congo *S. pneumoniae* was most frequent isolate from bacteriaemic SS patients (Eeckels 1967) and this organism was found in blood of all 8 cases with pneumonia in Northern Nigeria (Maharajan 1983). However, three other Nigerian studies (Akinyanju et al. 1987; Okuonghae et al. 1993; Akuse 1996) have shown a paucity of *S. pneumoniae*. None occurred among 19 bacteraemias in Lagos (Akinyanju and Johnson 1987), one among 54 bacteraemias in Benin City (Okuonghae et al 1993), and none among 304 children admitted with acute illness in Kaduna (Akuse 1996) although this organism was found in the CSF of 3 children. In Uganda there was also a death of *S. pneumoniae* as a cause of septicaemia (Etima et al. 2002, personal communication, Nantanda 2006 and Katureebe 2007, unpublished data). In five African studies from Nigeria and Uganda, the most common causes of bacteremia were *Klebsiella* spp., *Staphylococcus aureus*, and *Salmonella* spp. The virtual absence of *S. pneumoniae* in these studies is unexplained but could be due to the widespread use of across-the-counter antibiotics, death before presentation to hospital in patients septicemic with *S. pneumoniae*, or the intriguing possibility that malaria encourages persistence of splenic function in patients with SS disease (Akuse 1996). The hypothesis of greatest concern in those children with *S. pneumoniae* septicaemia is that they die before reaching hospital; although this seems intrinsically unlikely (three children with *S. pneumoniae* in the CSF reached hospital in Kaduna).

Since invasive disease with *S. pneumoniae* is believed to be secondary to nasopharyngeal carriage (Chesney 1992), studies of carriage rates may contribute to resolving this apparent conundrum. Carriage rates in US studies have been reported as 10 - 33% (Overturf et al 1980, Anglin et al. 1984; Norris et al. 1996; Steel et al. 1996) and have been reduced by regular penicillin (Anglin et al. 1984; Steel et al. 1996), but no data are reported from Africa. A further concern in the management of pneumococcal disease elsewhere, is the rapid emergence of penicillin resistance and preliminary observations suggest this to be common in Uganda (Najjuka 1995; Joloba et al. 2001) The present study has therefore addressed two objectives; the rate of nasopharyngeal carriage of *S. pneumoniae* in SS children attending a Sickle Cell Clinic in Mulago Teaching Hospital in Kampala.

Materials and Methods

Patients. The patients attended the Sickle Cell Clinic of Mulago Teaching Hospital, in Kampala and the study was restricted to clinically well subjects with SS disease aged under 6 years. The diagnosis of SS disease was based on an FSA₂ pattern on haemoglobin electrophoresis and characteristic haematology. A total of 81 subjects (51 male, 30 female) were recruited over a period of 4 months and of these 23 had been admitted within the previous 2 weeks, usually for treatment of low haemoglobin levels. Specimens were taken from nasopharynx from all patients and all parents/guardians were questioned on the use of antibiotics over the 4 proceeding weeks and for the history of pneumococcal immunization. All parents/guardians were given an explanation of the study and signed a consent form. The ethical approval was obtained from Mulago Hospital Research and Ethical Committee and Faculty of Medicine Research and Ethical Review Board.

Specimens. The nasopharyngeal specimens were taken off using a pre-packed sterile disposable calcium alginate fiber tipped aluminium applicator swab and this was done by a paediatrician on duty. The specimens were put in Stuart's transport medium and transported to Department of Medical Microbiology, Faculty of Medicine for culture. Culture and identification of *S. pneumoniae* was done according to standard methods (Balows et al. 200).

Isolation and Identification of S.pneumoniae. The collected specimens were immediately inoculated on 5% rabbit blood agar. All plates were incubated for 24-48 hours at 35-37° C in 5% Carbon dioxide. *S. pneumoniae* isolates were identified using typical colonial appearance, alpha-haemolysis, and gram-staining. Confirmatory tests included optochin sensitivity and bile solubility.

Antibiotic susceptibility tests were done using the following disks: Oxacillin, 1µg, Erythromycin, Ceftriaxone, Chloramphenicol, Co-trimoxazole, Rifampicin, Perfloxacin. Susceptibility was performed from bacterial suspension whose turbidity was equivalent to that of a Mcfarland of 0.5. Using a sterile swab which had been dipped in the organisms suspension and excess fluid drained off and streaked on to 5% rabbit blood Columbia Agar base. The antibiotic disks were then dispensed; plates incubated at 35-37° C for 24 hours in 5% Carbon dioxide. The reading and interpretation of the sensitivity/resistance patterns was according to Cormican MG and Jones RN, 2000 (formerly NCCLS, 1993). Control organisms (*Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 29522) were included in each set of tests as controls.

RESULTS

S. pneumoniae was found in 27/81 (33%) of the study group (Table 1), occurring in 2/23 (9%) of those with recent hospitalizations and in 25/58 (30%) of those without hospitalisation ($\chi^2 = 8.8, p=0.003$). Two subjects had received pneumococcal immunization and neither carried the organism. Antibiotic usage usually for persistent coughs was reported in 64/81 (79%) subjects (Table 2). It was reported that the following antibiotics had been given to the children - penicillin group, Trimethoprim-

Sulfamethoxazole, Chloramphenicol and Cephalexin in form of mainly syrups. Antibiotic sensitivity of isolated organism showed all to be penicillin resistant but sensitive to other commonly used antibiotics (Table 3).

Exclusion criteria were children above 5 years because these are usually stable in their health, those who were presented ailments particularly pneumoniae and those that lacked consent from parents/guardians.

DISCUSSION

Streptococcus pneumoniae remains the commonest cause of community-acquired pneumonia, adulthood meningitis, bacterial otitis media, sinusitis and bacteremia worldwide.

Virtually all-invasive pneumococcal disease follows nasopharyngeal carriage. Sickle cell anaemia is one of the major predisposing factors to pneumococcal disease. In this study a prevalence of 33% nasopharyngeal carriage of *S. pneumoniae* among sickle cell children attending a sickle cell clinic in Uganda was found. These findings are higher than those reported by others elsewhere. Daw et al (1977) found only 13% of the 312 children with sickle cell disease studied in the United States. Steel et al (1996) found a carrier rate of 12% only significant in children with sickle cell less than 2 years ($p < 0.0001$). Anglin et al (1984) found only 14.5% *S. pneumoniae* carriage in children with sickle cell disease. Skull et al (1999) found 54% (1048/1974) carriage rate in children in Northern Territory of Australia and 30% (312/1048) penicillin resistance. However, their study did not indicate whether any of children had sickle cell disease or not.

Our study showed that antibiotic use in the subjects during the previous four weeks did not significantly affect nasopharyngeal carriage ($p=0.93$). Antibiotic resistance of *S. pneumoniae* was found to be highest (100%) to the commonly used antibiotics in Kampala and the surrounding districts and these were mainly penicillin and Trimethoprim-Sulphamethoxazole). This is not surprising because there is excessive use of penicillin and penicillin-like antibiotics in the country as these are freely sold to whoever comes in pharmacies and drug shops with or without prescription.

High resistance against penicillin (92.9%) has been shown in *S. pneumoniae* by earlier studies done in Uganda (Najjuka 1995) among people of different age groups. In a study carried out in 2000, Joloba et al (2001) found high levels of resistance against penicillin (83.3%) in strains of *S. pneumoniae* isolated from normal children in Kampala, Uganda. In a study done in Zambia, among 260 children below the age of 6 years by Wolfson et al (1997) only 12.7% of *S. pneumoniae* isolates were found to be resistant to penicillin. The big gap of resistance among strains isolated in Uganda and Zambia could indicate the difference in the law enforcement practices on the use of antibiotics in the two countries.

We have detected an extraordinary high prevalence of penicillin of *S.pneumoniae* strains isolated from the nasopharynges of children with sickle cell disease. In Uganda no penicillin prophylaxis is given to children with SS like in some western world countries because of costs and compliance problems. Furthermore, immunization using the

heptavalent pneumococcal conjugate vaccine (PCV 7) in Uganda is not widespread yet. This study will serve as a signal to be used for the initial empirical treatment of infections frequently caused by *S. pneumoniae*, for example, meningitis, pneumonia and septicaemia. Prospective studies of the treatment of invasive infections due to penicillin resistant *S. pneumoniae* with bigger samples are urgently needed. Lastly, studies to establish the prevalence of SCD in children is equally urgently needed in Uganda as there is no data on SCD in Uganda.

ACKNOWLEDGEMENTS

We are greatly indebted to Prof. C. Ndugwa, Head Sickle cell Clinic, Department of Paediatrics, Faculty of Medicine, Makerere University and Mulago hospital management for the permission granted to us to conduct this study at the Sickle Cell Clinic. Our appreciation goes to the Department of Medical Microbiology, Faculty of Medicine for the support of the project with all the consumables used in the the study. Lastly but not least, the children with SCD and their parents/guardians without whose consent this study would not have been possible.

References

Anglin, D.L., J.D. Siegel, D.L. Pacini, and S.J. Smith, G. Adams and G.R. Buchman (1984). Effect of penicillin prophylaxis on nasopharyngeal colonization with *Streptococcus pneumoniae* in children with sickle cell anaemia. *J. Padiatr.* **104** (1): 18-22.

Akinyanju O, and A.O. Johnson. (1987). Acute illness in Nigerian children with sickle cell anaemia. *Ann Trop Paediatr*; **7**: 181-6.

Akuse RM. (1996). Variation in the pattern of bacterial infection in patients with sickle cell disease requiring admission. *J Trop Pediatr*; **42**: 318-23.

Baker, C.N., C. Thornberry and R.W.Hawkinson, (1983). Inoculum standardization in antimicrobial susceptibility tests; Evaluation of the overnight agar cultures and the Rapid Inoculum standardization system. *J. Clin. Microbiol.* **17**: 450-457.

Barrett-Connor E, (1971). Bacterial infection and sickle cell anaemia. *Medicine.* **50**: 97-112.

Cormican MG and Jones RN (2000). Re-evaluations of disk diffusion susceptibility testing interpretive criteria for lomefloxacin and norfloxacin using fluoroquinolone-resistant isolates. *Diagnostic Microbiology of Infectio dis*: 21(4) 227-230.

Cowan, S.T. and Steel. (1974). Bacterial infection and Sickle Cell Anaemia. *Medicine.* **50**: 97-112.

- Chesney PJ. (1992). The escalating problem of antimicrobial resistance in *Streptococcus pneumoniae*. *American J. Dis. Child.* **146**:912-916,
- D'Amato RF and L. Hochstein, (1982). Evaluation of rapid inoculum preparation method for agar disk diffusion susceptibility testing. *J. Clin. Microbiolog.* **15**: 282-285.
- Daw NC, J.A. Williams, W.C. Wang, G.J. Presbury, R.E. Joyner, S.C. Harris, Y. Davis, G. Chen and P.J. Chesney, (1997). Nasopharyngeal carriage of penicillin resistant *S pneumoniae* in children with SCD. *J. Paediatr.* **99 (4)**:E7.
- Garcia-Leoni M.E., E.P. Cercendao J.C.L. Rodeno and B.de Quiros, (1992). Susceptibility of *S. pneumoniae* to penicillin: A prospective microbiological and clinical study. *Clin. Infect. Dis.* **14**: 427-435.
- Gray, B. M., G.M. Converse and H.C. Dillon (1980). Studies of *S. pneumoniae* in infants. Acquisition, carriage, and infection during the first 24 months of life. *J. Infect. Dis.* **142**: 923-933.
- Hengongo, S., J.A. Williams, S. Harris, S.W. Day and W.C Wang (1997). Recurrent *S. pneumoniae* sepsis in children with SCD. *J. Paedriatr.* **130(5)**: 814-816
- Hulley BS and S.R. Cummings, (1988). Sample size for a descriptive study of a dichotomous variable. In: *Designing Clinical Research.* Baltimore, William and Wilkkins. Appendix 13E, pp. 220.
- Joloba M, C. Whalen, E. Fasola, et al. (2001). High prevalence of carriage of antibiotic resistant *S. pneumoniae* in children in Kampala, Uganda. *Intern. J. Antimicrobial Agents.* **17 (5)**: 395-400.
- Klugman, K.P. and I.r. Friedland. (1995). Antibiotic resistant pneumococci in paediatric disease. *Micro and drug resist.* **1**: 5-8.
- Lobel, J.S. and K.E. Bove (1982). Clinicopathologic characteristics of septicaemia in SCD. *Am. J. Dis. Child.* **136**: 543-547.
- Maharajan R, Flemming AF and Egler L. (1983). Pattern of infections among patients with sickle cell anaemia requiring hospital admission. *Nig J Paed*;10:13–17.
- Musher, D.M. (1992). Infection caused by *S. pneumoniae*. Clinical spectrum, pathogenesis, immunity and treatment. *Clin. Infect.Dis.* **14**: 801-809.
- Najjuka, C.F. (1995). Antibigram of *S pneumoniae* isolated in Mulago Hospital with special reference to penicillin resistance. M. Med. (Pathology) Dissertation, Makerere University , Kampala.

Okuonghae HO, MU Nwankwo, EC Offor. (1993). Pattern of bacteraemia in febrile children with sickle cell anaemia. *Ann Trop Paediatr*; **13**: 55-64.

Overturf, G.D., S. Lee and D.R. Powars (1980). Nasopharyngeal carriage of pneumococci in children with sickle cell disease. *Infect. Immun.* **28**: 1048-1050.

Powars, J. S., Weiss and L. Chan (1995). Pneumococcal septicaemia in children with sickle cell anaemia. Changing trend of survival. *J. Am. Med. Ass.* **245**: 1839-1842.

Robinson B. and R.J. Watson. (1966). Pneumococcal meningitis in SCD. *N. Engl. J. Med.* **274**: 1006-1008.

Rogers, D.W., S. Vaidya and G. R. Sergeant (1978). Early splenomegaly in homozygous SCD: An indicator of susceptibility to infection. *Lancet* **2**: 963-965.

Sergeant, G.R. (1989). *Sickle Cell Disease*. Oxford Press Publications. 11: 153-159.

Skull, S. A., T. Shelby-James, P.S. Morris, et. al. (1999). *Streptococcus pneumoniae* antibiotic resistance in Northern Territory children in day care. *J, paediatr.& child health.* **35(5)**: 466- 471.

Steel, R. W., R. Warriar, P. J. Unkel et al. (1996). Colonization with antibiotic-resistant *S. pneumoniae* in children with sickle cell disease. *J, Paediatr.* **128(4)**: 531-535.

Wolfson, A., R. Hulbner, A. Wasa et. al. (1997). Nasopharyngeal carriage of community acquired antibiotic resistant *S. pneumoniae* in Zambian paediatr. Population. *Bull. World health Organ.* **75(5)**: 453-462.

Table 1: Nasopharyngeal Carriage of *S. pneumoniae* by Gender

CARRIAGE	SEX		TOTAL (n=81)
	MALE(n=51)	FEMALE(n=30)	
POSITIVE	18	9	27
NEGATIVE	33	21	54
TOTAL	51	30	81

Table 2: Drug use in the Previous Four Weeks *S. pneumoniae* carriage

DRUGS		1	2	3	4	5	6	7	8	9
CARRIERS	27	9	7	5	1	1	2	1	1	0
NON-CARRIERS	54	19	12	3	4	3	4	5	2	1
TOTAL	81	28	19	8	5	4	6	6	3	1

KEY:

- 1- No Antibiotic used
- 2- Trimethoprim-sulfamethoxazole
- 3- Penicillin
- 4- Amoxicillin and Cloxacillin
- 5- Augmentin
- 6- Amoxicillin and Septrin
- 7- Ampicillin
- 8- Chloramphenicol
- 9- Cephalexin

Table 3: Summary of Antibiotic Susceptibility of all the Isolated Strains

ANTIBIOTIC	<i>S.pneumoniae</i> strains		
	SUSCEPTIBLE	INTERMEDIATE RESISTANT	RESISTANT
Penicillin (by Oxacillin 1µg screening)	0(0%)	-	27(100%)
Trimethoprim-sulfamethoxazole(23.75µg)	0(0%)	1(3%)	26(97%)
Chloramphenicol (30µg)	26(97%)	-	1(3%)
Erythromycin (25µg)	26(97%)	-	1(3%)
Rifampin (5µg)	27(100%)	-	0(0%)
Ceftriaxone (30µg)	27(100%)	-	0(0%)
Perfloxacin (17µg)	17(100%)	-	0(0%)