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Research Article

Human Respiratory Syncytial Virus Subtypes A and B Infection Among Children Attending Primary and Secondary Health Care Facilities in Ibadan, Nigeria

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Abstract

Human respiratory syncytial virus (HRSV) causes high morbidity and mortality in infants and young children. Although a high prevalence of HRSV has been reported in Nigeria, the subtype of the virus circulating in the country is not known. This cross-sectional study was therefore designed to determine the subtypes of HRSV circulating among children in Ibadan. Two hundred and thirty-one nasopharyngeal and oropharyngeal swabs were collected from children presenting with respiratory infections in Secondary Health Facility (SHF) as well as those attending immunization centers in Primary Health Centers (PHCs) in Ibadan, Nigeria. Viral RNA was extracted directly from the clinical specimen and used for HRSV detection with a pair of primers that targets the conserved region of the viral matrix gene. HRSV-positive samples were subtyped using subtype-specific primers targeting the second hypervariable region of the G gene. The prevalence of HRSV infection was 8.7% and 34.6% among children attending the PHCs and SHF respectively. Both subtypes of HRSV were detected (co-circulating) among the study population. None of children was co-infected with of HRSV A and B. Overall, HRSV-A was the predominant subtype detected among children presenting with respiratory infection at the SHF while subtype B was predominant among participants attending PHCs for routine immunization. Higher disease severity scores were associated with HRSV – A infection than infection with HRSV – B. Only HRSV subtype A was detected from those diagnosed of bronchopneumonia and bronchiolitis. In conclusion, subtypes A and B co-circulating among children in Ibadan, with HRSV – A being the predominant subtype. Additional study including samples collected from other parts of the country over a longer period that will cover both wet and dry season will be done to determine the pattern of HRSV circulation in Nigeria.

Key Words: *Respiratory Tract Infections, Lower Respiratory Infections, Children, Human Respiratory Syncytial Virus, Subtype*

INTRODUCTION

Lower Respiratory Infections (LRI) is the leading cause of mortality due to infectious disease both in Africa and globally, and the second leading cause of mortality in Nigeria (Global Burden of Diseases and Injuries in Children and Adolescents, 2017). A high proportion of LRI has been associated with Human Respiratory Syncytial Virus (HRSV) infection, especially in infants, young children and vulnerable adults (Falsey *et al*., 2005; Nair *et al*., 2010). The enveloped virus with single-stranded, non-segmented, linear RNA genome of negative polarity, approximately 15.2kb in size is the prototype Orthopneumovirus in the recently classified Pneumoviridae family (Human and Moore, 2016). Over 50% of children show serological evidence of HRSV infection by their first birthday and nearly all children are seropositive by two years of age (Shay *et al*., 1999) . The virus is known to cause repeated infection throughout life, perhaps due to immune response that does not last long (Beem, 1967; Glezen and Denny, 1973; Scott *et al*., 2007). The clinical presentations vary from mild upper respiratory tract infections (URTI) which may not require hospitalization to severe and life-threatening illness with lower respiratory tract involvement resulting in bronchiolitis and pneumonia that requires hospital care (Midulla *et al*., 2010; Tran *et al*., 2013) .

Although infections may occur all year round both in the temperate and tropical regions of the world, HRSV has been found to cause epidemics during the winter months in the temperate countries or during the rainy season in the tropical countries (Trento *et al*., 2006; Eshaghi *et al*., 2012; Aamir *et al*., 2013)

In spite of the high burden of infection due to HRSV in Nigeria (Robertson *et al*., 2004; Odaibo *et al*., 2013),, very little is known about the virus circulating and causing disease in the country. Previous studies in Nigeria described the prevalence, seasonality and severity of HRSV infection among different cohorts (Nwankwo *et al*., 1988; Olaleye *et al*., 1992; Johnson *et al*., 1993; Akinloye *et al*., 2011; Odaibo *et al*., 2013; Faneye *et al*., 2014) however the subtypes circulating are not established. Knowledge of the circulating virus subtype is important for prevention and control strategies including effectiveness of currently available as well as design of new vaccine for use in the country.

MATERIALS AND METHODS

Study location and population: Ibadan is the capital city of Oyo State, Nigeria and the third largest metropolitan area, by size and population in Nigeria. The city has 11 Local Government Areas (LGAs), consisting of five urban and six

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semi-urban LGAs. It has a tropical wet and dry climate, with a lengthy wet season that runs from March through October. Samples for this study were collected from March to October 2015 from children attending routine immunization clinics in two primary health centers (one each from an Urban and a semi urban LGAs) in Ibadan metropolis and showing signs of respiratory infection as well as from those seeking medical care due to respiratory infection at the Our Lady of Apostle Catholic Hospital, Oluyoro, a secondary health care facility in Ibadan. Some of the participants recruited at the Our Lady of Apostle Catholic Hospital were hospitalised, while others were seen at the outpatient clinic. Signs of respiratory infection were identified using WHO protocol for the identification of Influenza-like illnesses (ILIs) and/or Severe Acute Respiratory Infections (SARI) (WHO, 2005; WHO, 2009). Samples were collected from participant showing one or more of the signs of respiratory infection (cough, rhinorrhea, difficulty breathing, bronchiolitis or pneumonia, etc) with or without fever. Informed assent were obtained from parents / guardian and only children with whose onset of illness was within 7 days were included in the study. The UI/UCH ethics committee approved the study protocol with approval number UI/EC/14/0284.

Clinical samples. Both nasopharyngeal and oropharyngeal swabs were collected from 231 children. The samples were transported on ice to the Department of Virology, College of Medicine, University of Ibadan for further processing and appropriate storage. Where RNA extraction was not possible on same day of collection, samples were kept in -80oC until processed. Demographic details of the study participants were collected using structured questionnaire.

RT-PCR for HRSV detection: Viral RNA was extracted directly from the clinical specimen using RNAeasy Mini Viral RNA kit (Qiagen, Hilden Germany) according to manufacturer's instruction. cDNA was generated by reverse transcriptase reaction using random hexamer primers and SCRIPT reverse transcriptase (Jena Bioscience, Germany). Five microliters of the cDNA was used as template in a 25µl PCR reaction mix consisting of DNA Taq polymerase, dNTPs, KCl, and MgCl2. Detection of the presence of HRSV in the samples was done using a pair of primer (RSV Forward – GGCAAATATGGAAACATACGTGAA, RSV Reverse - TCTTTTTCTAGGACATTGTAYTGAACAG) that targets the conserved region of the viral matrix gene (Aamir *et al*., 2013). Amplification was carried out under the following conditions: 94oC for 2 min, followed by 40 cycles of 94oC for 30s, 53oC for 30s and 72oC for 45s and then a final extension at 72oC for 5min in a conventional PCR procedure. The expected amplified products of 84bp were detected in a 2% agarose gel by electrophoresis (Figure 1). HRSV A2 (ATCC-1540) obtained from National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG, WHO International Laboratory for Biological Standard, UK was used as positive control while PCR grade water was used as no template control in each step to validate the assay. HRSV-positive samples were selected for subtyping using HRSV – A and HRSV – B specific primers as previously described (Aamir *et al*., 2013).

Statistical analysis of epidemiological factors: Chi square was used to test the level of significance of differences in proportions of various demographic and clinical features and p-value of < 0.05 was considered statistically significant

RESULTS

The ages of the children included in the study ranged from 1 month and 72 months (mean = 9.76 months). Children, aged 0 - 6 months constituted the majority [128 (55.4%)] of the study participants (Table 1). Most participants [150 of 231 (65%)] in this study presented at the Primary Health care facilities (Table 1)

Table1:
Detection of HRSV among the study participants.

	Number tested	Number Positive	P - value
Level of Health Facility			<0.01
PHC	150	13 (8.7)	
SHF	81	28 (34.6)	
Total	231	41 (17.7)	
Gender			>0.05
Male	120	23 (19.2)	
Female	111	18 (16.2)	
Age Group			0.002
0 – 6 months	128	15 (11.7)	
7 – 12months	74	13 (17.6)	
13 – 24months	14	6 (42.9)	
>24months	15	7 (46.7)	

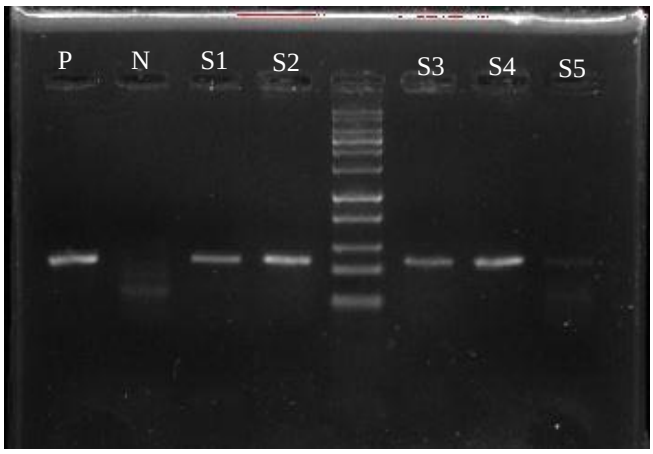

Figure 1:
Electrophoresis image of positive samples in 2% agarose gel**.** Low range DNA Ladder (Jena Bioscience, Germany) was used to detect the expected DNA size. PC= Positive Control, NC= Negative Control, S1 – S5 were test samples.

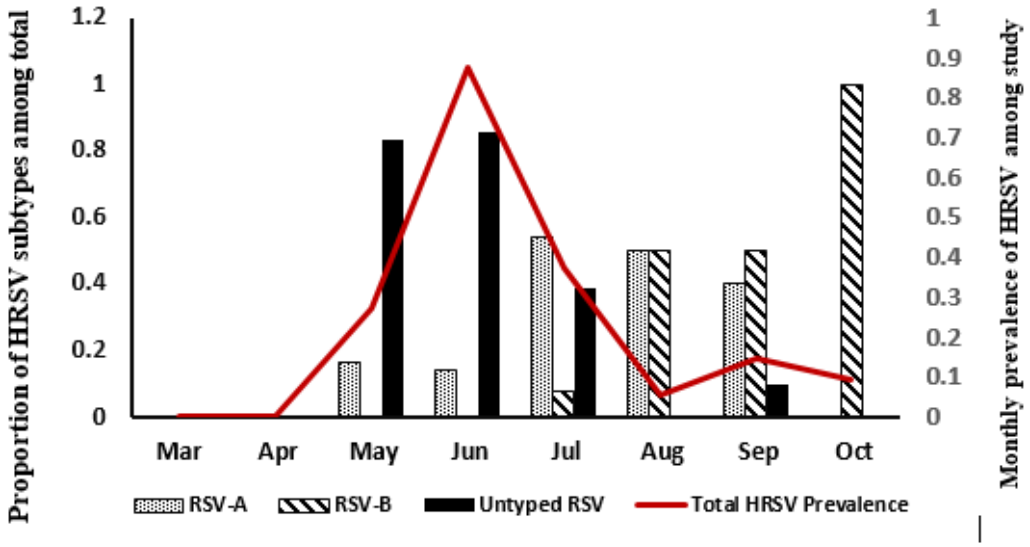

Figure 2:
Distribution of Human Respiratory Syncytial Virus (HRSV) A and B by month of sample collection. The proportion of the subtypes in the HRSV positive samples are represented by the bars. The prevalence of HRSV are represented by the histogram plot.

Table 2:
HRSV subtypes detected in Ibadan, Nigeria

	HRSV SUBTYPES			P value
	No Tested	Subtype A	Subtype B	
Health facility				
Primary	13	2 (15.4)	10 (76.9)	<0.001
Secondary	28	20 (71.4)	1 (3.6)	
Total	41	22 (53.7)	11 (26.8)	

Table3:
Disease severity scoring by HRSV types.

SEVERITY SCORE	HRSV SUBTYPE P>0.05 n (%)		
	No Tested	**HRSV-A**	**HRSV - B**
3	19	12 (63.2)	4 (21.1)
4	12	5 (41.7)	4 (33.3)
6	2	2 (100.0)	0 (0.0)
7	1	0 (0.0)	1 (100.0)
8	1	1 (100.0)	0 (0.0)
9	4	1 (25.0)	0 (0.0)
11	2	2 (100)	0 (0.0)
Total	41	23 (56.1)	9(22.0)

A higher severity score indicates more severe disease.

The overall prevalence of HRSV infection was 17.7% (Table 1) while the peak of HRSV activity was found in the month of June (Figure 2). The male to female ratio among the study participants was approximately 1:1 and there was no significant difference in the rate of HRSV infection in both gender (p=0.558). Both subtypes of HRSV were detected and found co-circulating children attending primary healthcare facilities for routine immunization and those presenting with respiratory infection for care at the secondary health facilities. Co-infection with HRSV A and B was found in any of the study participants. The predominant subtype detected among children attending Secondary Healthcare Facility was HRSV-A while HRSV-B was the predominant subtype among participants for routine immunization at Primary Healthcare Facilities (Table 2). The higher severity scores were associated with HRSV – A infection than infection of HRSV – B (Table3). All the HRSV detected from those that presented with bronchopneumonia and bronchiolitis were subtype A (Table 4).

Table4:
Symptoms observed in the study participants.

Symptoms	**RSV - positive N (%)**	**RSV - negative N (%)**	**P - value**
Cough	41 (100)	161 (84.7)	0.007
Wheezing	5 (12.2)	3 (1.6)	0.001
Apnea	7 (17.1)	13 (6.8)	0.035
Fever	24 (58.5)	90 (47.4)	0.195
Nasal congestion	2 (4.9)	56 (29.5)	0.001
Rhinorrhea	34 (82.9)	164 (86.3)	0.574
Clinical Diagnosis			
Bronchopneumonia	4 (100.0)	0 (0.0)	
Bronchiolitis	1 (50.0)	1 (50.0)	

DISCUSSION

Both subtypes of HRSV (i.e. A and B) detected and found to be co-circulating among under 5 years old children attending primary and secondary healthcare facilities in Ibadan. The participants attending primary health centers (PHCs) were considered apparently healthy by their parents / guardians and were only brought to the health center to receive vaccine. On the other hand, those enrolled at the secondary health facility (SHF) where children with one form of respiratory illness or the other purposely brought to the hospital for care. Expectedly, the prevalence of HRSV among children enrolled

at the SHF (34.6%) was significantly higher than those enrolled at the PHCs (8.7%). However, the relatively high prevalence of the infection among children attending PHCs for routine vaccination brings to fore the importance of Immunization Clinics in the transmission of HRSV among children below 1 year in Nigeria, considering the high turnout of children in clinics during such immunization days. Similar prevalence was reported in Kenya among children attending outpatient's clinics (Okiro *et al*., 2012). Overall, the HRSV prevalence of 17.7% was found among the study participants. This shows that HRSV is still a significant cause of respiratory infection in Nigeria as earlier reported (Olaleye *et al*., 1992; Robertson *et al*., 2004; Odaibo *et al*., 2013) The rate of HRSV found in this study is similar to the that reported among children less than 5 years old in Kenya (Bigogo *et al*., 2013) . Although HRSV subtype A was the predominant subtype detected in the study and some other countries such as Vietnam (Tran *et al*., 2013), Kenya (Scott *et al*., 2004) and Philippines (Malasao *et al*., 2015), evidence available in the literature shows that the predominant subtype alternate from time-to-time (Peret *et al*., 1998; Mlinaric-galinovic *et al*., 2009; Mlinaric-galinovic *et al*., 2012; Dong *et al*., 2015). Interestingly, subtype B was the predominant subtype detected among children attending PHCs for routine immunization. This supports the findings of Tran *et al*. (2012) who reported that subgroup B infected children were admitted to the hospital less often than subgroup A infected children. The fact that subtype A was predominant children with respiratory infection attending the SHF for care shows that HRSV A is more likely to cause more severe respiratory illness and is more associated to hospital visits and admission than HRSV B. The HRSV subtype in 3 of the 7 participants showing severity score ≥ 8 could not be ascertain, the available subtype data suggest that infection with subtype A resulted in more severe disease than that of subtype B. This inference was corroborated by the result of the HRSV subtype detected among children clinically diagnosed of bronchopneumonia and bronchiolitis. Our finding agrees with most studies that subtype A of HRSV is associated with more severe clinical disease (Mufson *et al*., 1988; Taylor *et al*., 1989; McConnochie *et al*., 1990; Walsh *et al*., 1997; Tran *et al*., 2013). All the children with clinical diagnosis of bronchiolitis and bronchopneumonia from whom HRSV were detected were 12 months and below, with 3 of the 4 (75%) being below 6 months of age. This is similar to the finding by Lamarao *et al*. (2012), and further lends credence to available evidence in the literature (D´Elia *et al*., 2005; Siqueira *et al*., 2005; Lee *et al*., 2007; Midulla *et al*., 2010; Piedimonte and Perez, 2014; Sricharoenchai *et al*., 2016) suggesting that RSV is the main pathogen responsible for bronchiolitis and pneumonia during the first 12 months of life. Illness resulting from HRSV infection in this age group is also known to be severe (Lamarao *et al*., 2012).

Unlike most studies where the highest prevalence was reported in younger age groups (Weber *et al*., 1998; Tran *et al*., 2013; Fall *et al*., 2016), we found here that the prevalence of HRSV increased with age. This may be because of the disparity in the number of children in the different age groups enrolled for the study. Although the prevalence was slightly higher in male children, gender was not significantly associated to susceptibility to HRSV infection in our study and is similar to reports by other researchers (Aamir *et al*., 2013; Tran *et al*., 2013).

In conclusion, subtypes A and B of HRSV were found co-circulating among children attending Primary healthcare facilities for routine immunization and Secondary Health Facilities to receive for respiratory illness in Ibadan, Nigeria. Subtype A, the predominant HRSV among children presenting with respiratory illness may cause more severe illness than HRSV subtype B Additional study that with samples collected across both the wet and dry seasons and from other parts of the country is required to determine the pattern of HRSV circulation in Nigeria.

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