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Research Article

Incidence and Burden of Respiratory Syncytial Virus Infection in a Community-Based Cohort of Under-Five Years Children in Nigeria

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Abstract
Respiratory Syncytial Virus (RSV) is one of the most common causes of lower respiratory tract infection (LRI) in children under 5 years. Most of the available epidemiological information on RSV infection are from developed countries where denominator based studies have been done. We hereby describe our findings in a WHO sponsored study that estimated the incidence of the RSV infection in children in urban and rural communities in Nigeria. The study was designed as a prospective, population-based cohort of under-five children in an urban (Eleta) and a rural (Ijaiye) community in Oyo State, Nigeria. Nasopharyngeal wash was collected from each child with LRI into sterile plain 5mls tubes and transported daily to the laboratory on ice. An aliquot of each specimen was tested for presence of RSV antigen using an EIA and another aliquot inoculated into Hep2 cell line for virus isolation. Data analyses were performed using the EPIINFO version 6.0. Frequencies were compared using chi-square test at 95% confidential level and incidence reported as per 1000 child years. A total of 2,015 children were enrolled for the study among which 413 episode of LRI occurred. The overall incidence of RSV associated LRI during the 2 years of follow-up was 125/1000 child years. The incidence of RSV in Ijaye was 1.6 times (CI, 0.31 – 1.2) and 1.9 times (CI, 0.9 – 3.6) higher than that of Eleta in the first year and second year respectively. The highest incidence of RSV infection occurred among the age group 3-5 months in Eleta and the age group 9-11 months in Ijaiye. No gender preponderance in the incidence of RSV was observed. This study provided for the first time, a denominator based prevalence and incidence of RSV at the community level in Nigeria. The rates of RSV among under-five children in rural and urban communities in Nigeria are high.

Keywords*: *Respiratory Syncytial Virus, Incidence, Prevalence, Rural and Urban communities, Nigeria

Introduction

Respiratory syncytial virus (RSV) is a major cause of childhood morbidity and mortality throughout the world. It is one of the most common causes of lower respiratory tract infection (LRI) in children under 5 years (Robertson *et al*., 2004, Bryce *et al*., 2005, Nair *et al*., 2010). It is highly contagious, being shed in respiratory secretions for several days, sometimes for weeks and easily spread by direct contact and droplets from the nose. Nosocomial infection and outbreaks of RSV in related institutions are common occurrence (Herderson *et al*., 1979). In institutions such as day care settings the attack rate may be up to 100% (Herderson *et al*., 1979; Simoes, 2003). Most children become infected in their first year or two and reinfections occur throughout life (Nair *et al*., 2010, Weber *et al*., 1998).

RSV accounts for about 50% of all cases of pneumonia and up to 90% of all reported cases of bronchiolitis during infancy in some places (Weber *et al*., 1998). The virus has been shown to infect about 65% of infants during their first year of life and about one-third of those who develop LRI (Nair *et al*., 2010; Robertson *et al*., 2004). Nair *et al*. (2010) estimated that at least 33.8 million RSV associated acute LRI occurred among under 5 years old children worldwide in 2005. By two years of age, virtually all children in some parts of the world are infected with RSV and almost 50% with multiple episodes (Weber *et al*., 2002; Hacmustafaoglu *et al*., 2013).

While RSV infection had been reported from industrialized and developing countries, significant information on the epidemiology of the virus in the literature are based on studies from the developed countries. In most parts of the developed world, epidemics of RSV are predictable and the burdens as well as severity of the problem are known. Hence it is possible to plan effective preventive and control measures ahead of epidemics. On the other hand, in the developing countries, particularly in Africa, most information are derived from reports and data from hospital-based studies (Robertson *et al*., 2004). Although some reports indicate that the incidence of RSV in the developing countries appear to be similar or even higher than in the developed countries (Nair *et al*., 2010; Simoes 1999), the total burden of RSV associated illness and other epidemiologic factors required for planning of preventive and control measures against the virus have not been well documented.

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In Nigeria, although a few hospital-based studies have documented the importance of RSV in the aetiology of LRI (Nwankwo *et al*., 1988; Olaleye *et al*., 1992), the epidemiological data on the virus available in the country are scanty and inconsistent. The inconsistency between the available data is probably due to lack of defined denominator for proper epidemiologic interpretation or comparison of the results of those previous studies. In this report, we describe the results of the first part of a WHO sponsored two-year, prospective community-based study on the epidemiology of RSV-induced LRI in two cohorts of under-five children in Nigeria.

Materials and Methods

Study design and sites

The study was a prospective, population-based cohort of under-five children in an urban (Eleta) and a rural (Ijaiye) community in Oyo State, south west of Nigeria. A WHO generic protocol (Wright and Cutts, 2000) by the steering committee on Epidemiology and Field Research was used as the reference for the project. Ethical approval for the study was obtained from the University of Ibadan/University College Hospital IRB as well as the Oyo state MOH Ethical Committee.

The study was carried out in two stable communities and the plan was discussed with the traditional and opinion leaders of the sites before its commencement and during the period of the study. The urban site, Eleta is a community within the city of Ibadan with an estimated population of 10,000 people from the 1991 national census figure. It is a typical high density Nigerian urban community with several households per house. It is located within the central area of Ibadan city, the largest and most populous indigenous city in Nigeria with over 6 million people. The community is bothered by a stream on the west and major roads on the other sides provide the boundaries. The community is serviced by a primary health care centre (PHC) and a missionary hospital. Occupation of the people includes petty trading, artisans, transportation and a few civil servants. Eleta is 5km away from the Virology Laboratory of the University College Hospital, Ibadan where the laboratory component of the study was based. The second site, orile-Ijaye is a rural community about 20km NW of Ibadan with an estimated population of 11,302 from the 1991 national census figures. The community has predominantly mud houses with some cement plastering and entirely with iron sheet roofing but mostly no ceiling. The place is serviced by a PHC which is the only government health centre and the main occupations of the people are farming and trading.

The residents of both communities are predominantly Yoruba tribe of indigenous Ibadan ethnic clan/ group. The two sites have also been used by UNICEF and the University College Hospital, Ibadan for health-related projects in the past. A house to house baseline enumeration of children <5 years was conducted and the houses were assigned numbers in both communities. Following informed consents of the parents, children <5 years of age and subsequent newborns in each house were registered by household and assigned a card with study number and a study booklet with the child's identification number for the follow-up visits. Children reaching their 5th birthday were excluded from the study. Migration in and out of both communities was minimal.

Case ascertainment

Cases of LRI in the communities were identified by active surveillance conducted by qualified Nurses and Community Health Workers (CHW). They were engaged full time and trained for the project in the recognition of LRI, collection of nasal aspirates and transportation to the laboratory using the standard methods as outlined in the WHO generic protocol for the study (Wright and Cutts, 2000). Some of the Nurses and CHWs lived in the communities to ensure that cases that occurred outside the daily working hours and weekend were not missed. Study households were visited daily and experienced Consultant Pediatricians supervised the health care workers and monitored case ascertainment and diagnosis.

Case of LRI and the definition of episode of LRI were according to the WHO generic protocol for the study (Wright and Cutts, 2000). The generic signs of LRI are age dependent. Briefly, for children below 2 months of age, signs of LRI were fast breathing (<_60/minute) or severe chest in-drawing or stridor in a calm child or wheezing or apnea. For children 2-59 months, fast breathing (>_50/minute in a child age 2-11 months and <_40/minute in a child 12-59 months) or chest in drawing or stridor in a calm child or wheezing or apnoea (Wright and Cutts, 2000). Each child's study booklet was completed weekly during the weekly home visits and case report forms specifically designed for the project were completed for each LRI detected. Severely ill children were referred to the University College Hospital, Ibadan for appropriate management and admission where necessary.

Inclusion criteria for the study were residents in the communities, under 5 years of age and parental assent. Incentives for participation in the study were provision of some medication such as cough syrup, paracetamol, multivites and Vitamin C as well as payment of other bills associated with management of case on behalf of the patients.

Specimen collection and laboratory methods:

Nasopharyngeal wash using 2ml sterile PBS was collected from each child with LRI into sterile plain 5mls tubes at the patient's house by a trained nurse. The specimen tubes were stored in a cold box with ice packs immediately after collection and transported daily to the laboratory in the Department of Virology, University College Hospital, Ibadan for registration and processing.

One aliquot of the specimen was tested for presence of RSV antigen. A second aliquot was inoculated into two tubes of monolayer of Hep2 cells for virus isolation (to be described in another paper) while the remaining specimen was frozen with and without RNASOL in cryovials for genotyping later.

The Sanofi Diagnostics Pasteur Inc. (Pathfinder TH) RSV antigen detection EIA kit (cat #79674; Lot/ch-B G019; Lot/ch-B 9H020; Lot/ch-B OM037; Lot/ch-B OE032) was used to detect presence of RSV antigen in the nasal washing from each child who presented with LRI as well as from culture supernatants. The method is based on use of both polyclonal and monoclonal antibodies to detect RSV antigens directly in the specimens. The assay uses

polyclonal anti-RSV antibodies which reacts with RSV antigen along with peroxidase-conjugated monoclonal antibodies specific for an epitope on the RSV nucleocapsid antigen. Any sample with a positive Narsopharyngeal aspirate or culture was considered as positive for RSV.

Statistical Analysis

Data analyses were performed using the EPIINFO software package version 6.0 (CDC, Atlanta, USA). Data from each child's study booklet, case report form and laboratory tests were entered using the ENTER module of the program. Both interactive and back checking of data (during and after respectively) were done using the CHECK and VALIDATE modules of the program. Frequencies were compared using chi-square test at 95% confidential level. Incidence was calculated using the formula (Number of new infection ÷ population at risk X 1000) and reported in per 1000 child year (Scott *et al*., 2013).

Results

A total of 2015 under 5-year-old children were included in the study cohort (1332 in urban and 683 in rural community) over the 2 years follow-up period (1999 – 2001). Less than 10% of children dropped out of the study mainly because they attained the age of 5 years (60 months). Table 1 and 2 shows the monthly distribution of the children during the follow-up period in the rural and urban community respectively.

Four hundred and thirteen episodes of LRI occurred over the period (168, urban and 245, rural) out of which 146 (35.4%) were positive for RSV either by culture or antigen capture EIA. The sensitivity of virus detection by direct ELISA was higher than virus culture. One hundred and forty-two (34.4%) of ELISA positive were culture negative while 3.2% of ELISA negative were positive in culture. The overall incidence of RSV associated LRI during the 2 years of follow-up was 125/1000 child years. The incidence did not vary significantly between the first and second year of follow-up in both communities (Table 3).

There was however a slight drop in the incidence during the 2nd year in both communities. Further analysis showed that the incidence of RSV in Ijaye was 1.6 times (CI, 0.31 – 1.2) and 1.9 times (CI, 0.9 – 3.6) higher than that of Eleta in the first year and second year respectively.

Table 1:
Monthly child weeks of follow-up by age groups in Eleta (Urban), Nigeria

Month	1st Year							2nd Year						
	Age Groups (Months)							Age Group (Months)						
	0-2	3-5	6-8	9-11	12-23	24-59	Total	0-2	3-5	6-8	9-11	12-23	24-59	Total
June	28	21	23	20	81	130	**303**	242		103	143	604	984	2383
July	63	44	61	39	188	221	**616**	250	217	220	173	730	1146	2837
Aug.	76	39	56	50	244	261	**726**	281	278	212	152	690	1070	2660
Sept.	85	48	74	47	252	326	**832**	263	255	200	147	653	1031	2535
Oct.	106	62	99	62	320	395	**1044**	326	241	236	175	787	1232	3046
Nov.	118	77	108	68	320	349	**1040**	282	290	212	147	850	1057	2799
Dec.	207	214	186	160	682	1032	**2481**	307	251	237	171	759	1135	2884
Jan.	264	266	246	197	775	1250	**2998**	373	275	272	201	831	1185	3181
Feb.	228	222	208	169	679	1101	**2607**	321	319	233	168	895	995	2891
March	241	234	229	188	718	1143	**2753**	383	279	252	202	761	11047	2944
April	186	237	222	182	695	1118	**2640**	426	299	275	227	859	11170	3298
May	275	265	246	216	793	1254	**3049**	409	341	266	216	930	1210	3373
Total	**1877**	**1729**	**1758**	**1398**	**5747**	**8580**	**21573**	**3903**	**342**	**2808**	**2122**	**13262**	**13262**	**34831**

Table 2:
Monthly child weeks of follow-up by age groups in Orile-Ijaiye (Rural), Nigeria

Month	1st Year							2nd Year						
	Age Groups (Months)							Age Group (Months)						
	0-2	3-5	6-8	9-11	12-23	24-59	Total	0-2	3-5	6-8	9-11	12-23	24-59	Total
June	14	25	30	34	89	155	**347**	183	104	101	122	409	512	**1431**
July	37	59	62	66	246	364	**834**	202	124	103	126	388	483	**1426**
Aug.	74	61	80	89	286	434	**1024**	225	144	114	136	428	497	**1544**
Sept.	113	69	76	81	290	417	**1046**	193	120	102	118	364	430	**1327**
Oct.	155	83	88	90	317	470	**1203**	216	129	114	136	412	590	**1597**
Nov.	151	90	96	97	300	463	**1197**	156	115	96	114	377	424	**1282**
Dec.	186	110	99	116	372	556	**1439**	193	105	90	113	353	390	**1244**
Jan.	150	82	81	111	308	459	**1191**	222	124	107	124	410	439	**1426**
Feb.	201	88	83	109	303	426	**1210**	190	107	87	108	347	349	**1188**
March	192	100	100	116	341	448	**1297**	208	107	90	112	374	369	**1260**
April	213	95	97	109	316	420	**1250**	206	114	90	116	355	352	**1233**
May	249	211	118	133	354	471	**1536**	221	127	104	122	411	382	**1367**
Total	**1735**	**1073**	**1010**	**1151**	**3522**	**5083**	**13574**	**2415**	**1420**	**1198**	**1447**	**4628**	**5217**	**16325**

Table 3:
Incidence in Per 1000 Child Year (CI at 95%)

	Year 1	Year 2	Total	p
Ijaiye	130 (88.4 – 171.6)	125 (88.4-166.4)	129.8	0.87
Eleta	78.0 (52.0 – 104.0)	64.2 (41.6– 78.0)	70.5	0.29
RR	1.6 (0.31-2.2)	1.85 (0.9-3.6)	1.73	

RR=Rate Ratio
Overall incidence = 125/1000 child years

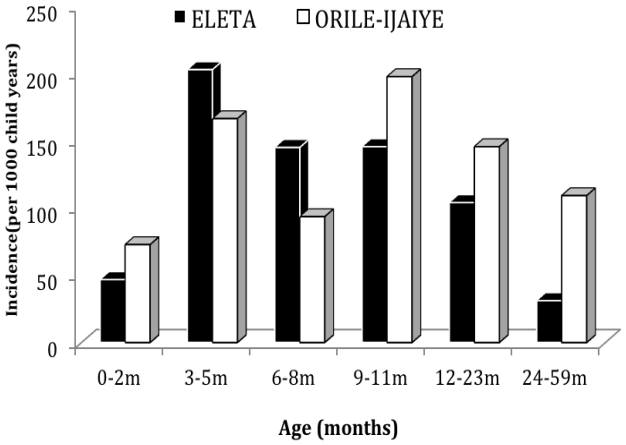

Figure 1:
Incidence of RSV in Eleta and Ijaiye communities by age group (combined years 1 and 2)

RSV was detected among all the age groups. Figure 1 shows the incidence of RSV (per 1000 child week) by age of the children in the urban and rural communities during the 2 year period. In Eleta, the urban community, the highest incidence of RSV infection (202.8/1000 child year) was observed among the age group 3-5 months in the 2 years. In the rural community, Ijaye, the age distribution pattern was slightly different with the highest incidence (196.6/1000 child year) of RSV infection occurring among the age group 9-11 months. However, the rate was also high among those in the 3-5 months age group (166.4/1000 child years). The lowest incidence was found among the oldest children (24-59 months) in Eleta and the youngest (0-2 months) in Ijaiye communities (figure 1), though the incidence at this young age group in Ijaiye was high when compared with same group from Eleta. Fifty- five percent of the children enrolled for the study were female while 45% were male but there was no significant gender preponderance of RSV infection in the two communities. The male to female ratio RSV associated LRI was 48:52.

Seventeen children (4.1%) with LRI had multiple episodes of RSV infection during the follow-up period. Multiple episode is defined as infection in the same individual that occurs two or more weeks apart. The interval between infections in these individuals ranged from 4 weeks to 7 months. Sixteen cases had 2 episodes of RSV infection while one child had the infection up to 3 times. Blood samples from the children with multiple RSV infection were tested for HIV infection and all were negative.

In all, 35 deaths (8.7 per 1000 child year), 26 in urban and 9 in the rural cohorts occurred during the two years period. Thirteen (37.1%) of these deaths were LRI related out of which 3 (8.6%) were RSV related, giving an RSV associated mortality rate of 7.4 per 10,000 child year in the study communities Nigeria.

Discussion

The Overall incidence of RSV for the two years of study was 124.8/1000 child years. As far as it can be ascertained, this is the first incidence data on RSV in Nigeria. Most of the RSV incidence information available in the literature are from developed countries and very little is known about the situation in Africa. The incidence of 124.8/1000 child years reported here indicate a very high burden of RSV infection among children <5 years of age in Nigeria. This rate is higher than recent rates reported in Kenya (90/1000 child year) (Nokes *et al*., 2008) but similar to rates in Australia with incidence of 110-226 per 1000 child year (Ranmuthugala *et al*., 2011). However, lower incidence rates have been reported in some other developed countries (Moura *et al*, 2003)

The prevalence rate of 35.4% found among our cohort is higher than reports from previous studies in the country (Nwankwo *et al*, 1988; Olaleye *et al*., 1992). About 10 years before this study, Olaleye *et al*. (1992) reported an overall RSV infection rate of 23.5% among hospitalized children in Ibadan. The technique used in the 1992 study, CFT antibody conversion testing may be a less sensitive assay compared with the antigen detection test used in this study and hence the difference in the RSV rate in the two studies. On the other hand, the higher rate of RSV infection may be an indication of increase in the incidence of RSV infection over time since no vaccine against the infection is available in the country till date. It has also been suggested that difference in RSV rates may be due to climatic conditions, environmental factors as well as severity of RSV epidemics from one year to another (Medici *et al*., 2004; Wang *et al*., 1996). However, the rate is close to the 31.9%, 35.8%, 31.4% report in Italy (Medici *et al*., 2004) Thailand (Kaneko *et al*., 2002;) and Japan (Suwanjutha *et al*., 2002) respectively.

There was no significant difference between the overall incidence in the first and second year of the study. Thus signifying the endemicity of RSV infection in Ibadan, Nigeria. The incidence was however consistently higher in the rural than in the urban area during the 2 years. This suggests that there may be some environmental or social factors that potentiate the spread of RSV infection in such rural communities. The incidence obtained in the rural community is one of the highest ever recorded, next only to data from Cal-Cambodia, Alaska and Thailand which are countries with extreme temperatures (Borrero *et al*., 1990, Karron *et al*., 1999, Kaneko *et al*, 2002). Because of the severity and frequent complication of viral infection caused by super-infection with bacteria, the high incidence obtained is very worrisome especially in an environment with very poor health system such as rural settings in Nigeria.

The pattern of distribution of RSV infection by age varied in the rural and urban community. In Eleta, the highest incidence was among the age group 3-5 months in the 2 years. On the other hand, the peak of infection in Ijaye was among children 9-11 months though with also high level of infection in the 3-5 months age group. This difference is in support of an earlier finding in The Gambia which showed that the rate of occurrence of RSV is dependent on area or living condition (Weber *et al*, 2002). The findings of this study are also in agreement with reports that over half of children are infected during their first year of life (Heilman, 1990; Hall *et al*., 1990). Similarly, another study from Ibadan showed that all children with RSV associated bronchiolitis were not more than 12 months of age (Akinloye *et al*., 2011).

The multiple episode rate of 4.1% obtained among children with LRI in our cohort is lower than findings of Medici *et al*. (2004) who reported multiple RSV episode in 28.1% of their cohort in Italy. Their study was however hospital based and this may have accounted for the great difference. Some factors such as immunodeficiency and malnutrition have been associated with multiple RSV infection episodes (Hall *et al*., 1986; Nwankwo *et al*., 1994; Chalab, 2013). We investigated the role of HIV, a major cause of immunodeficiency in African children (UNAIDS, 2010) in the occurrence of multiple episode of RSV infection and showed HIV infection was not a predisposing factor for multiple episode as none of these children tested positive to HIV. The mortality due to RSV infection of 8.6% was found in this study. This rate is higher than the 0.5 to 4.0% rate reported among the same population group in Italy (Medici *et al*., 2004). Nair *et al*. (2010) estimated that 66,000 to 199,000 children younger than 5 years died from RSV associated ALRI in 2005; out of which 99% occurred in the developing country.

This study also provided opportunity to compare two techniques in diagnosis of RSV infection, direct antigen capture from nasopharyngeal aspirate versus culture. Over one-third (34.4%) of the nasopharyngeal aspirate that were positive by EIA did not yield virus in tissue culture while 3.2% of samples that yielded virus in culture were initially negative by direct antigen capture EIA. Although direct antigen capture from NA seems to be more sensitive, few cases of RSV infection may still be missed with this technique. Combination of the two techniques would be more reliable for diagnostic purpose in centres where necessary facilities are available. In a similar study in Taiwan, out of 892 Nasopharyngeal aspirate tested, 775 were positive for RSV antigen by IFA, 239 by culture and only 122 were positive by both techniques (Lee *et al*, 2007). The time for completion of the direct antigen capture EIA however, gives it an added advantage over the other methods (Hendry *et al*., 1985). It has been suggested that the extreme thermo-liability of RSV as well as the fact that culture can only detect viable virus makes the use of virus isolation an inappropriate tool for estimation of viral burden or incidence (Nokes *et al*., 2008).

In conclusion, the results of this denominator based study show a high burden of RSV infection among under-five children in Nigeria, with higher incidence among children less than one year old. These findings further emphasize the need for use of RSV vaccine for effective prevention and control of the virus infection, especially in infants.

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Conflict of interest:
We declare that we have no conflicting interest in the conduct of the study.

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