Supplementary appendix

Related Healthcare Burden: A Prospective Observational Study in a resource-constrained setting

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Supplementary text descriptions of methods

Text S1. Inclusion criteria for the active RSV surveillance in the IPD

Children aged 0–59 months admitted to the selected wards were evaluated by study physicians using the WHO RSV hospital-based surveillance case definition (13). Briefly, <5-year children hospitalized with a respiratory infection, defined as having cough or shortness of breath, with an onset within the last 10 days, were eligible to be enrolled in the study. In addition, infants younger than six months were also eligible if they presented with apnoea, which is characterized by a temporary cessation of breathing from any cause and/or sepsis (defined as fever (temperature of 37.5 °C or above) or hypothermia (temperature less than 35.5 °C), with indications of shock (lethargy, fast breathing, cold skin, prolonged capillary refill, or a fast, weak pulse) and seriously ill with no apparent cause.

Text S2. Nasopharyngeal swab collection and qPCR methods

Nasopharyngeal swab samples were collected by trained nurses using mini flocked swabs (FLOQSwab, Copan Diagnostics, California, USA) and were immediately stored in 1 ml skim milk tryptone-glucose-glycerol (STGG) media. RNA was extracted from 140 µl of the sample volume using the Qiagen QIAamp® Viral RNA Mini kit on QIAcube Connect system (Qiagen, Hilden, Germany). Subsequently, 7 µl of extracted RNA underwent qPCR with qScript XLT 1-Step RT-qPCR ToughMix (QuantaBio, Beverly, MA, USA) and previously reported RSV-specific primers and probe. Final concentrations of primers and probe were 900 nM and 200 nM, respectively, in a 20 µl qPCR reaction volume. qPCR was conducted on Applied Biosystem qPCR 7500 Fast Dx system (Thermo Fisher Scientific, Waltham, MA, USA), with cycling conditions were 50°C for 10 minutes, 95°C for 1 minute, and 40 cycles of 95°C for 10 seconds and 60°C for 1 minute. RSV-positive cases were identified based on true sigmoidal amplification curves and a cycle threshold (Ct) <35.

Forward primer: - 5’- GGCAAATATGGAAACATACGTGAA-3’

reverse primer: 5’-TCTTTTTCTAGGACATTGTAYTGAACAG-3’

probe: 5’-CTGTGTATGTGGAGCCTTCGTGAAGCT-3’ (labeled at the 5’ end with FAM and quenched at the 3’end with Black Hole Quencher-1).

Text S3. Monte Carlo Simulation

We estimated the daily average number of cases requiring admission by month. We also estimated the distribution of hospital stay lengths by fitting a negative binomial distribution to our observed data for patients over 5 years who were admitted. Daily admissions were simulated using Poisson sampling from these daily rates. Cases were either admitted or refused based on bed availability, with admitted cases assigned hospital durations based on draws from the fitted negative binomial distribution. When beds were limited, cases were randomly selected for admission. Each case was randomly assigned an outcome (death within 90 days) based on the standardized cumulative survival probability estimated from the adjusted multivariate Cox Proportional Hazards model in our data (1). The survival probability differed between admitted and refused patients. The simulation progressed day by day, updating the number of free beds daily based on admissions and the length of stay of previously admitted cases. We ran simulations for the baseline scenario, and for different efficacies of RSV vaccines where specific numbers of cases were reduced from the daily rates of presenting and requiring admission. For example, a 100% reduction scenario meant subtracting all RSV cases in our data to estimate the daily average rates. Since we lacked RSV test results for refused cases, we assumed the proportion of RSV cases among the refused was the same as among the admitted. We simulated a range of hospital bed capacities and vaccine efficacies and present results for simulated changes to 90-day mortality for the baseline RSV burden and a 70% reduction in burden. In the absence of real-world vaccine effectiveness data, our simulation was based on the Phase 3 clinical trial in which the Pfizer pre-F fusion vaccine (Abrysvo) reduced the risk of severe lower respiratory tract disease caused by RSV by 82% at three months and 69% at six months after birth(1,2). Considering the age distribution in our study and imperfect vaccine coverage, a 70% reduction in burden could be a realistic scenario. Each scenario was run with 1000 iterations; each iteration lasted for 380 days, but only the last 365 days were used for analysis to ensure a steady state of daily refusals.

Supplementary tables

Table S1. Assigned clusters of related diagnoses.

Respiratory manifestation	Pneumonia/ bronchopneumonia
	Severe pneumonia
	Bronchiolitis/ acute bronchiolitis
	ARI/Acute respiratory infection
	RDS/ Respiratory distress Syndrome
Systemic infections	Septicaemia / sepsis
	Neonatal sepsis
Febrile illness	Enteric fever/ Typhoid fever / Paratyphoid fever
	Febrile convulsion / Atypical febrile convulsion
	Viral fever/Dengue Fever
Neurological manifestation	Meningitis
	Encephalitis/Meningoencephalitis/Encephalopathy
	Seizure disorder/Neonatal seizure
	Epilepsy
Gastrointestinal manifestation	Acute gastroenteritis/ AGE/Acute watery diarrhea
	Persistent diarrhoea
	Dysentery/ Shigellosis/ Invasive diarrhoea
Genitourinary/Renal manifestation	Nephrotic syndrome
	Renal failure/ kidney failure
	AGN/ Acute Glomerulonephritis/ APSGN
Cardiovascular manifestation	Ventricular septal defect/ VSD
	Tetralogy of Fallot/ TOF
	Other congenital heart disease
Perinatal asphyxia	Perinatal asphyxia
Preterm low-birth weight	Preterm low-birth weight
Neonatal jaundice	Neonatal jaundice
Others	Others

Table S2. Reasons for sample collection failure

Reasons of no sample collection	Number of Cases	Percentage
Patients' refusal to participate	152	14.6
Failure in sample collection	6	0.6
Use of oxygen mask	133	12.8
Being in a special ward	64	6.1
Discharged before specimen collection	130	12.5
Being enrolled respectively	496	47.6
Other reasons	61	5.9

Table S3. Time of sample collection. Time of sample collection was available for 6119 of 6149 samples.

Difference between time of admission and sample collection	Number of Samples	Percentage
0-23 hours	2,732	44.6
24-47 hours	2,161	35.3
48-71 hours	717	11.7
72+ hours	509	8.3

Table S4. Reasons for lost of follow-up at 14 days (refused children), n = 493

Reasons	Number of Cases	Percentage
Wrong number	84	17.0
Phone switched off	273	55.4
Did not receive call	133	27.0

Consent refusal	3	0.6

Table S5. Reasons for lost of follow-up at 90 days (refused children), n = 300

Reasons	**Number of Cases**	**Percentage**
Phone switched off	209	69.7
Did not receive call	65	23
Missing data	22	7.3

Table S6. Reasons for lost of follow-up at 14 days (admitted children), n = 323

Reasons	**Number of Cases**	**Percentage**
Wrong number	53	16.4
Phone switched off	168	52.0
Did not receive call	92	28.5
Others/Missing	10	3.1

Table S7. Reasons for lost of follow-up at 90 days (admitted children), n = 337

Reasons	**Number of Cases**	**Percentage**
Phone switched off	68	20.2
Did not receive call	21	6.2
Others/Missing	248	73.6