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Severe outcomes associated with respiratory viruses in newborns and infants: a prospective viral surveillance study in Jordan

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ABSTRACT

Objective To assess virus-specific hospitalisation rates, risk factors for illness severity and seasonal trends in children hospitalised with acute respiratory infections (ARI).

Design Prospective cohort study.

Setting A government hospital serving low-income and middle-income population in Amman, Jordan.

Participants Children under 2 years of age hospitalised with fever and/or respiratory symptoms (n=3168) from 16 March 2010 to 31 March 2013. Children with chemotherapy-associated neutropenia and newborns who had never been discharged after birth were excluded from the study.

Outcome measures Hospitalisation rates and markers of illness severity: admission to intensive care unit (ICU), mechanical ventilation (MV), oxygen therapy, length of stay (LOS) and death.

Results Of the 3168 subjects, 2581 (82%) had at least one respiratory virus detected, with respiratory syncytial virus (RSV) being the most predominant pathogen isolated. During admission, 1013 (32%) received oxygen therapy, 284 (9%) were admitted to ICU, 111 (4%) were placed on MV and 31 (1%) children died. Oxygen therapy was higher in RSV-only subjects compared with human rhinovirus-only (42%vs29%, p<0.001), adenovirus-only (42%vs21%, p<0.001) and human parainfluenza virus-only (42%vs23%, p<0.001) subjects. The presence of an underlying medical condition was associated with oxygen therapy (adjusted OR (aOR) 1.95, 95% CI 1.49 to 2.56), ICU admission (aOR 2.51, 95% CI 1.71 to 3.68), MV (aOR 1.91, 95% CI 1.11 to 3.28) and longer LOS (aOR1.71, 95% CI 1.37 to 2.13). Similarly, younger age was associated with oxygen therapy (0.23, 95% CI 0.17 to 0.31), ICU admission (aOR 0.47, 95% CI 0.30 to 0.74), MV (0.28, 95% CI 0.15 to 0.53) and longer LOS (aOR 0.47, 95% CI 0.38 to 0.59). Pneumonia was strongly associated with longer LOS (aOR 2.07, 95% CI 1.65 to 2.60), oxygen therapy (aOR 2.94, 95% CI 2.22 to 3.89), ICU admission (aOR 3.12, 95% CI 2.16 to 4.50) and MV (aOR 3.33, 95% CI 1.85 to 6.00). Virus-specific hospitalisation rates ranged from 0.5 to 10.5 per 1000 children.

Conclusion Respiratory viruses are associated with severe illness in Jordanian children hospitalised with ARI. Prevention strategies such as extended breast feeding, increased access to palivizumab and RSV vaccine development could help decrease hospitalisation rates and illness severity, particularly in young children with underlying medical conditions.

Strengths and limitations of this study

- ► This 3-year prospective surveillance study uses real-time reverse-transcriptase PCR to test a cohort of over 3000 children for 11 major respiratory viruses.
- ► To our knowledge, this study is the first to report virus-specific hospitalisation rates and illness severity in Jordanian children hospitalised with acute respiratory infections (ARI).
- ► We fit a multivariable logistic model to analyse the risk factors for severe illness, and used a Bayesian hierarchical model to derive the population prevalence of each respiratory virus.
- ► This study is limited to children from low-income and middle-income population seeking care at a single government hospital, potentially underestimating the true burden of ARI in Jordan.

INTRODUCTION

Acute respiratory infections (ARI) are the leading cause of death beyond the neonatal period in children under 5 years of age worldwide.[1] Historically, bacterial pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type B have been noted as predominant aetiologies[1–3]; however, respiratory viruses are frequently implicated as primary causes as well. A recent study conducted in the US found that respiratory viruses were much more common in children hospitalised with pneumonia than bacteria, with at least one virus detected in 65% of enrolled children.[4] A multicentre study conducted in four developing countries also noted that even though *S. pneumonia* was associated with increased risk of pneumonia deaths,

respiratory viruses were frequently associated with severe, hypoxic pneumonia.[5 6]

Earlier studies have estimated that there were 66 000–199 000 respiratory syncytial virus (RSV)-related deaths in 2005 and 28 000–1 115 000 influenza-related deaths in 2008 in children under 5; however, these systematic reviews did not include countries in the Middle East due to the paucity of population-based studies in the region.[7 8] The seasonality of respiratory viral diseases other than RSV and influenza and their overall burden in low-income and middle-income countries also remain poorly defined.[9] Additionally, prior published viral surveillance studies from the region are limited by their retrospective nature, the use of convenience samples over short time periods and the lack of use of modern molecular techniques such as real-time reverse-transcriptase PCR (RT-PCR).[10 11] Thus, the true burden and severity of virus-driven ARIs in Middle Eastern children are less defined.

We conducted a prospective surveillance study that tracked ARI-associated hospitalisations over a 3-year period among Jordanian children. The objectives of this study were to estimate virus-specific hospitalisation rates, identify risk factors for illness severity and characterise seasonal variations of respiratory viral infections in Jordan.

METHODS

Study design and participants

In this prospective surveillance study, we enrolled children under 2 years old who were hospitalised with fever and/or respiratory symptoms between 16 March 2010 and 31 March 2013 at Al-Bashir Hospital in Amman, Jordan (detailed inclusion/exclusion criteria previously reported).[12] Subjects were recruited 5 days a week (Sunday through Thursday) within 48 hours of hospital admission.[12] Children with chemotherapy-associated neutropenia and newborns who had never been discharged after birth were excluded from the study.

Written informed consent was obtained from parents or guardians.

Setting

Al-Bashir Hospital is one of the three major government hospitals in Amman, which is Jordan's largest city and capital.[12] We estimate that during the study period, the hospital provided care for ≈55% of all children in Amman (author Samir Faouri (2014), unpublished data). Additionally, the paediatric wards admitted 17 557 children, 11 230 (64%) of whom were under 2 years of age.

Data and specimen collection

After consent, trained local research staff obtained blood either by heel stick or venipuncture, as well as nasal and throat swabs. Standardised questionnaires were used to record demographic, clinical and socioeconomic data. Parents were queried in Arabic, and bilingual research staff transcribed the information into an English-language case report form at the time of the interview. After discharge, charts were abstracted for the following: antibiotic use, blood, urine, and cerebrospinal fluid (CSF) cultures, chest radiography, oxygen use, intensive care unit (ICU) stay, mechanical ventilation (MV), length of stay (LOS) in the hospital and discharge status.

All data were entered into a standardised, secure REDCap (Research Electronic Data Capture, Vanderbilt University, Nashville, Tennessee, USA) database.[13] We performed data quality checks on a minimum of 10% of the charts and verified data from all case report forms after entry.

Variables

Admission to ICU, oxygen therapy, MV placement, LOS and death were considered markers of severe illness. ICU admission included children who were transferred in during their hospital stay or were admitted directly. Smoke exposure included both cigarette and/or hookah pipe (nargila) smokers in household. Underlying medical conditions (UMCs) were classified as the following: diabetes, heart disease, Down syndrome, kidney disease, sickle cell disease, cystic fibrosis, cancer, genetic/metabolic, cerebral palsy, neurological, mental retardation/developmental delay, seizure disorder, chronic diarrhoea (eg, >2 weeks), gastro-oesophageal reflux disease, immunodeficiency, asthma/reactive airway disease, liver disease or other. Subjects were categorised as suspected sepsis if they had the admission diagnosis of rule-out sepsis or febrile neonate. Covariates included age, gestational age at enrolment, birth weight, sex (male), smoke exposure, breastfeeding status, UMC, vitamin D levels, viral detection, pneumonia, sepsis, bronchiolitis and bronchopneumonia. Cycle threshold (Ct) values were used as a proxy for viral load, ie, lower Ct values indicated higher viral load.

Laboratory testing

Nasal and throat swabs were collected and combined in transport medium (M4RT, Remel, USA), aliquoted into MagMAX Lysis/Binding Solution Concentrate (Life Technologies, USA), snap-frozen and then stored at −80°C. Aliquots were shipped on dry ice to Nashville, Tennessee, USA, and were tested by RT-PCR for 11 viruses: RSV; human rhinovirus (HRV); human metapneumovirus (HMPV); influenza (influenza) A, B and C; parainfluenza virus (PIV) 1, 2 and 3; adenovirus (AdV) and Middle East respiratory syndrome coronavirus (MERS-CoV).[14–16] Blood was placed directly onto filter paper and air dried for ≥30 min before storage at room temperature and kept dry through shipment to ZRT Laboratory (Beaverton, Oregon, USA) for 25(OH)D (vitamin D) level measurement (techniques published previously).[17]

Statistical analysis

Descriptive statistics were presented as frequency (percentage) or median and IQR where appropriate.

Categorical variables were compared using Pearson χ^2 tests. Continuous variables were compared using Mann-Whitney U test. For comparisons of continuous variables for ≥3 groups, Kruskal-Wallis tests were used. We fit a multivariable logistic model to analyse the risk factors for oxygen therapy, ICU admission, MV or longer LOS. Risk factors included breast feeding, vitamin D level, age at enrolment, gestational age, sex, UMC, smoke exposure (both cigarette and hookah), viral detection and four admission diagnoses (pneumonia, suspected sepsis, bronchiolitis and bronchopneumonia) based on literature review.[18] All analyses were performed using statistical software R V.3.1.2 (http://www.R-project.org/). Bonferroni adjustments were made to account for multiple comparisons in univariate analyses.

Models for each virus were fitted using Markov chain Monte Carlo.[19 20] Models were run for 100 000 iterations, with the first 90 000 iterations conservatively discarded as burn-in. Models were checked for convergence using the Gelman-Rubin diagnostic.[21 22]

Rate calculation

Al-Bashir Hospital admissions data were used to estimate the population prevalence of each respiratory virus. These data were filtered to exclude admissions of individuals not residing in greater Amman. We used a Bayesian hierarchical model to derive estimates for each of the 3 years of the study.[21] Estimates of the under 2-year-old Jordanian population were obtained from the World Bank online database, and the proportion of the population residing in Amman (35%) was taken from the 2012 national census. These values were used in a binomial model to estimate the population of children <2 years of age in greater Amman in 2010–2012. A binomial data likelihood was specified:

$$y^{v}_{a,t} \sim Binomial\left(n_{a,t}, p^{v}_{a,t}\right)$$

where $y^{v}_{a,t}$ is the number of children of age a in year t at Al-Bashir observed with virus v, while $n_{a,t}$ is the population of children age a in year t eligible for sampling, and $p^{v}_{a_t}$ the prevalence of virus v among children age a in year t. The sampled population $n_{a,t}$ was similarly estimated from a binomial model that sampled from $N_{a,t}$ the total Amman population of age a in year t:

$$n_{a,t} \sim Binomial\left(N_{a,t}, \pi\right)$$

Here, π is the proportion of the Amman population that is eligible to be sampled, which accounts for the market share m and the sampling intensity (5 days out of 7):

$$\pi = m\left(5/7\right)$$

The market share for Al-Bashir Hospital was modelled as a random variable, and given a uniform prior distribution between 50% and 60%, based on prior information. Prevalence was given diffuse beta(1,5) priors for all models. Note that the model structure implies all individuals carrying the virus in question seek hospital care.

Patient and public Involvement

Patient and public were not involved in this study design or input.

RESULTS

Study population

From 16 March 2010 to 31 March 2013, we screened and confirmed eligibility for 3793 patients. Of the 3793 patients, 618 had parent/guardian refusal, 3 were determined to be ineligible after enrolment due to age and 4 were diagnosed with meningitis. Our final sample consisted of 3168 subjects.

Demographics and clinical characteristics

The median age was 3.5 months, (range 0.07–23.96 months), 60% were male, 12% had UMCs and 14% were premature (<37 weeks gestation) (table 1). Of the 375 subjects with UMCs, heart disease was the most common, 146/357 (39%). Nearly 90% of the children's parents self-identified as Jordanian, and 7% reported Palestinian as their nationality. Primary and secondary education was the highest attainment in 41% and 44% of the mothers, respectively. The median birth weight was 3.0 kg, and 28% were born by caesarean section. The median number of siblings was 2, 1.6% attended daycare and 77% were exposed to smoke (73% and 18% to cigarette and hookah, respectively).

Before hospitalisation, 41% of the children had received antibiotics, and 92% were administered an antibiotic during their hospital stay (table 1). The seven main admission diagnoses included: bronchopneumonia (32%), suspected sepsis (28%), bronchiolitis (17%), pneumonia (12%), pertussis-like cough (7%), asthma/reactive airway disease (5%) and febrile seizure (3%). The median LOS was 5 days, with 9% admitted to the ICU, 4% on MV and 32% receiving oxygen therapy (table 1). Of the 2688 (85%) subjects who were tested for vitamin D levels, 49% had vitamin D deficiency (<20 ng/mL). The median level was 16.5 ng/mL (IQR 5.2–26) (table 1). During the study period, 31 (1%) children died (table 1). Among the 31 subjects, 21 (68%) had at least one respiratory virus detected (eight HRV positive; five RSV positive; one AdV positive; one influenza positive; and six coinfection cases).

Viral detection

At least one virus was detected in 2581 (81.5%) of the 3168 children. RSV (1397, 44%) was the most common virus detected, followed by HRV (1238, 39%), AdV (475, 15%), HMPV (273, 9%), PIV1–3 (175, 6%) and influenza A–C (119, 4%) (figure 1A). MERS-CoV was not detected in any sample. Viral codetection was common, with 944/2581 (37%) having at least one other virus codetected, ranging from 48% with RSV to 78% with AdV (figure 1A). Single virus detection (ie, excluding the children with viral codetection) was 728 for RSV only, 541 for HRV only, 106 for AdV only, 128 for HMPV only, 84 for PIV1–3 only and 50

Table 1 Univariate and multivariable analysis of factors associated with length of stay (LOS), risk of death, oxygen therapy, intensive care unit (ICU) admission and mechanical ventilation

	All (n=3168)	Virus negative (n=587)	Virus positive (n=2581)	>1 Virus (n=944)	RSV only† (n=728)	HRV only (n=541)	AdV only (n=106)	HMPV only (n=128)	PIV 1–3 only (n=84)	Influenza A-C only (n=50)
Age (months)‡	3.5 (1.6, 8.5)	2.4 (1.1–7.8)	3.8*** (1.8–8.6)	3.9 (1.8–8.4)	3.4 (1.7–7.1)	3.3 (1.6–8.6)	6.1 (1.9–12.1)	6.1*** (2.9–9.8)	5.3 (1.9–11.1)	6.6 (1.7–14.7)
Sex (male)	1912 (60)	357 (61)	1555 (60)	568 (60)	433 (59)	332 (61)	69 (65)	77 (60)	52 (62)	24 (48)
Breast feeding	2661 (84)	494 (84)	2167 (84)	794 (84)	627 (86)	444 (82)	91 (86)	103 (80)	68 (81)	40 (80)
No underlying medical condition	2793 (88)	502 (86)	2291 (89)	851 (90)	665 (91)	458 (85)***	93 (88)	113 (88)	70 (83)	41 (82)
Smoke exposure	2425 (77)	451 (77)	1974 (76)	720 (76)	558 (77)	411 (76)	82 (77)	98 (77)	65 (77)	40 (80)
Antibiotics prior to hospitalisation	1286 (41)	187 (32)	1099 (43)***	420 (44)	339 (47)	174 (32)***	48 (45)	66 (52)	31 (37)	21 (42)
Antibiotics during hospitalisation	2891 (92)a	532 (91)[b]	2359 (92)[c]	872 (93)	659 (91)	488 (91)	96 (91)	119 (94)	79 (94)	46 (92)
Preterm birth	450 (14)	88 (15)	362 (14)	141 (15)	89 (12)	80 (15)	15 (14)	20 (16)	12 (14)	5 (10)
Oxygen therapy	1013 (32)[d]	136 (23)[e]	877 (34)***[f]	331 (35)	305 (42)	152 (29)***	22 (21)***	39 (31)	19 (23)***	9 (18)
Mechanical ventilation	111 (4)[g]	22 (4)[e]	89 (3)[h]	30 (3)	29 (4)	20 (4)	3 (3)	2 (2)	4 (5)	1 (2)
Any ICU stay	284 (9)[i]	66 (11)[e]	218 (9)[j]	86 (9)	63 (9)	53 (10)	4 (4)	4 (3)	5 (6)	3 (6)
LOS (days)‡	5 (3–7)[k]	5 (3–8)[e]	5 (3–7)[l]	5 (3–7)	5 (3–7)	5 (3–8)	4 (2–7)	4 (3–6)	5 (3–8)	5 (3–6)
Death	31 (1)[m]	10 (2)[n]	21 (1)[o]	6 (1)	5 (1)	8 (1)	1 (1)	0 (0)	0 (0)	1 (2)
Vitamin D level (ng/mL)‡	16.5[p] (5.2–26.0)	16.0[q] (6.0–25.6)	16.7[r] (5.0–26.0)	15.8 (4.4–25.9)	15.3 (3.7–25.6)	18.5*** (7.9–26.2)	19.9 (6.7–31.3)	16.8 (8.6– 26.3)	17.9 (7.9–26.3)	17.3 (4.1–25.7)

Data are n (%) unless otherwise specified.
***P<0.001; calculated using Pearson χ^2 test for categorical variables and Wilcoxon rank-sum test for continuous variables.
†RSV only was the reference group for comparison between viruses.
‡Median (IQR).
an=3147; [b]n=586; [c]n=2561; [d]n=3137; [e]n=585; [f]n=2552; [g]n=3136; [h]n=2551; [i]n=3140; [j]n=2555; [k]n=3139; [l]n=2554; [m]n=3136; [n]n=583; [o]n=2553; [p]n=2688; [q]n=506; [r]n=2182.
AdV, adenovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

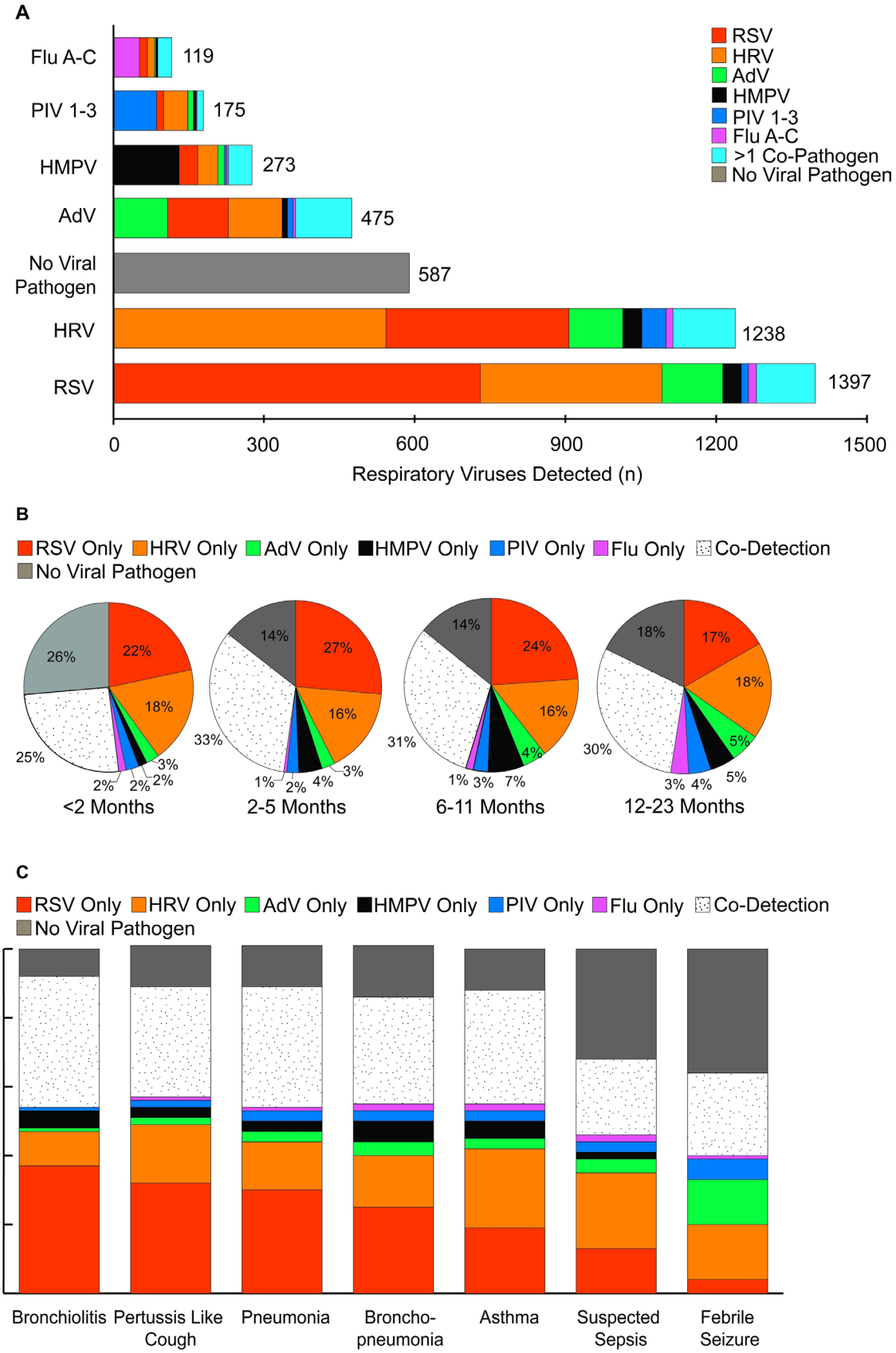

Figure 1 The viral pathogens detected in our Jordanian surveillance study. (A) The total number of pathogens detected by individual virus, with codetection of viral pathogens within each virus. (B) The proportion of viral pathogens detected by age group by single infection, coinfection and no viral pathogen. (C) The proportion of individual virus detection, coinfection and no viral pathogen by admission diagnoses. AdV, adenovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

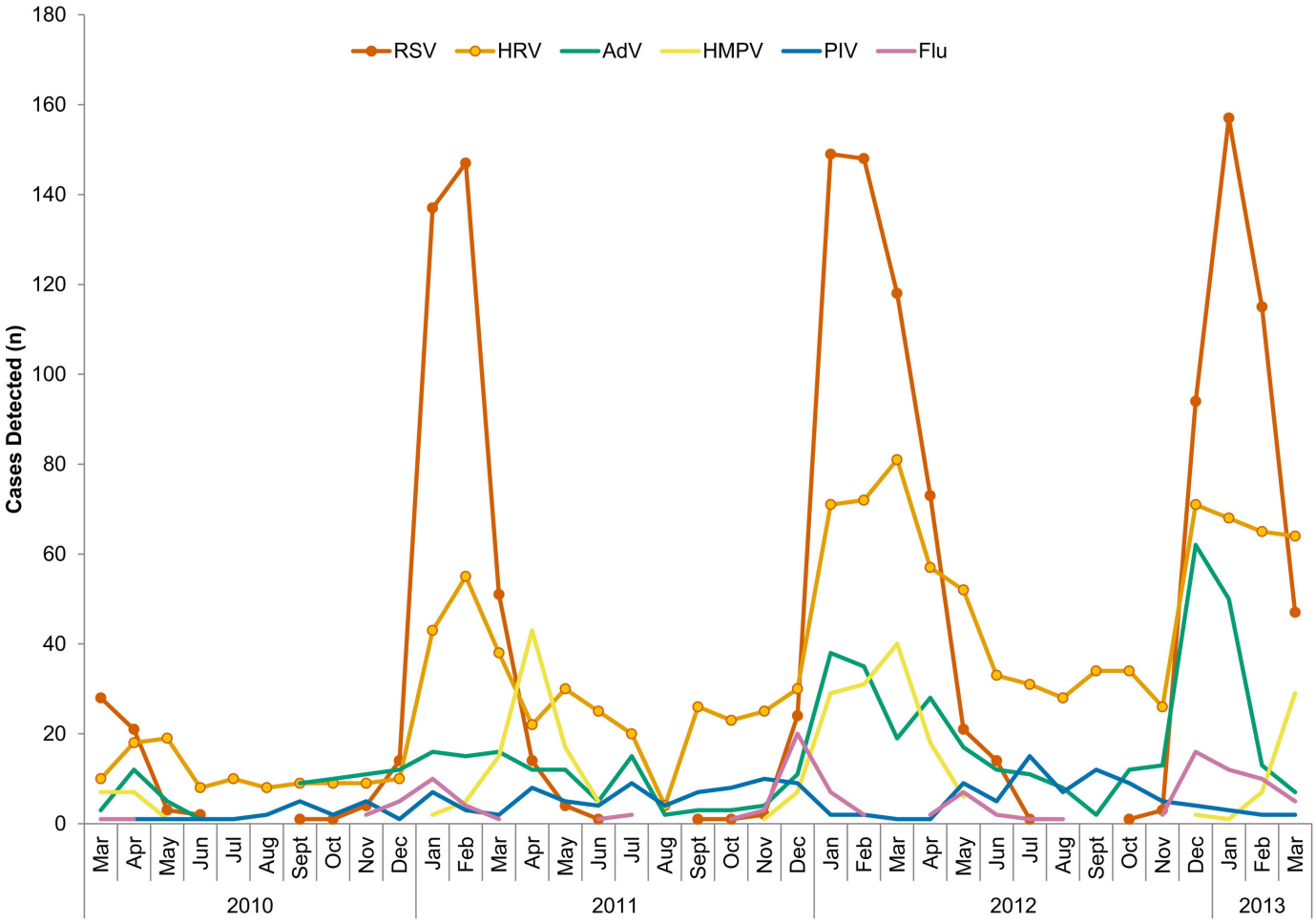

Figure 2 The distribution of the total number of viruses detected over 3 years by individual month in Amman, Jordan. AdV, adenovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; PIV, parainfluenza virus 1, 2 and 3; RSV, respiratory syncytial virus.

for influenza A, B and C only. Comparison of Ct values for single virus detection with codetection revealed a slightly higher viral load for single detection for RSV (25.2 vs 26.4, p<0.001); HRV (30.1 vs 31.5, p<0.001); HMPV (29.0 vs 32.0, p<0.001) and PIV (28.8 vs 32.4, p<0.01) cases. No significant differences in Ct values were seen for influenza and AdV cases. Figure 1B shows the viral distribution by age, with viral codetection common in all age groups. The percentage of viral detection by admission diagnoses is displayed in figure 1C. The frequency and distribution of all viruses over the 3-year period are displayed in figure 2, with >95% viral detection in the winter months, predominately RSV.

Bacterial detection

Reports of blood, urine and CSF cultures were available for 764, 769 and 614 subjects, respectively. Of these subjects, 38/764 (5.0%) had positive blood cultures, 118/769 (15.3%) had positive urine cultures and 4/614 (0.7%) had positive CSF cultures. Of the 150 subjects who tested positive for at least one bacterial culture, 104 (69.3%) also had viral codetection.

Hospitalisation rates

Of our 3168 subjects, 3048 (96.2%) resided in Amman. The highest rates of hospitalisation were due to RSV in years 1 (7.8/1000 children) and 2 (8.4/1000 children) and HRV in year 3 (9.6/1000). Hospitalisation rates were higher in children under 6 months old for all viruses compared with the older age groups, and those who were 6–11 months had higher rates compared with 12–23 months, based on non-overlapping credible intervals (figure 3).

Clinical outcomes

Virus-positive children were significantly more likely to require oxygen therapy (34% vs 23%, p<0.001) and receive an antibiotic prior to admission (43% vs 32%, p<0.001) (table 1) compared with virus-negative subjects. They were also more likely to present with cough (82% vs 42%, p<0.001) and shortness of breath (63% vs 34%, p<0.001); have wheezing (60% vs 37%, p<0.001), flaring (45% vs 24%, p<0.001) and cyanosis (21% vs 14%, p<0.001) on physical examination; and have the diagnoses of bronchopneumonia (34% vs 23%, p<0.001), bronchiolitis (19% vs 7%, p<0.001), and pneumonia (14% vs

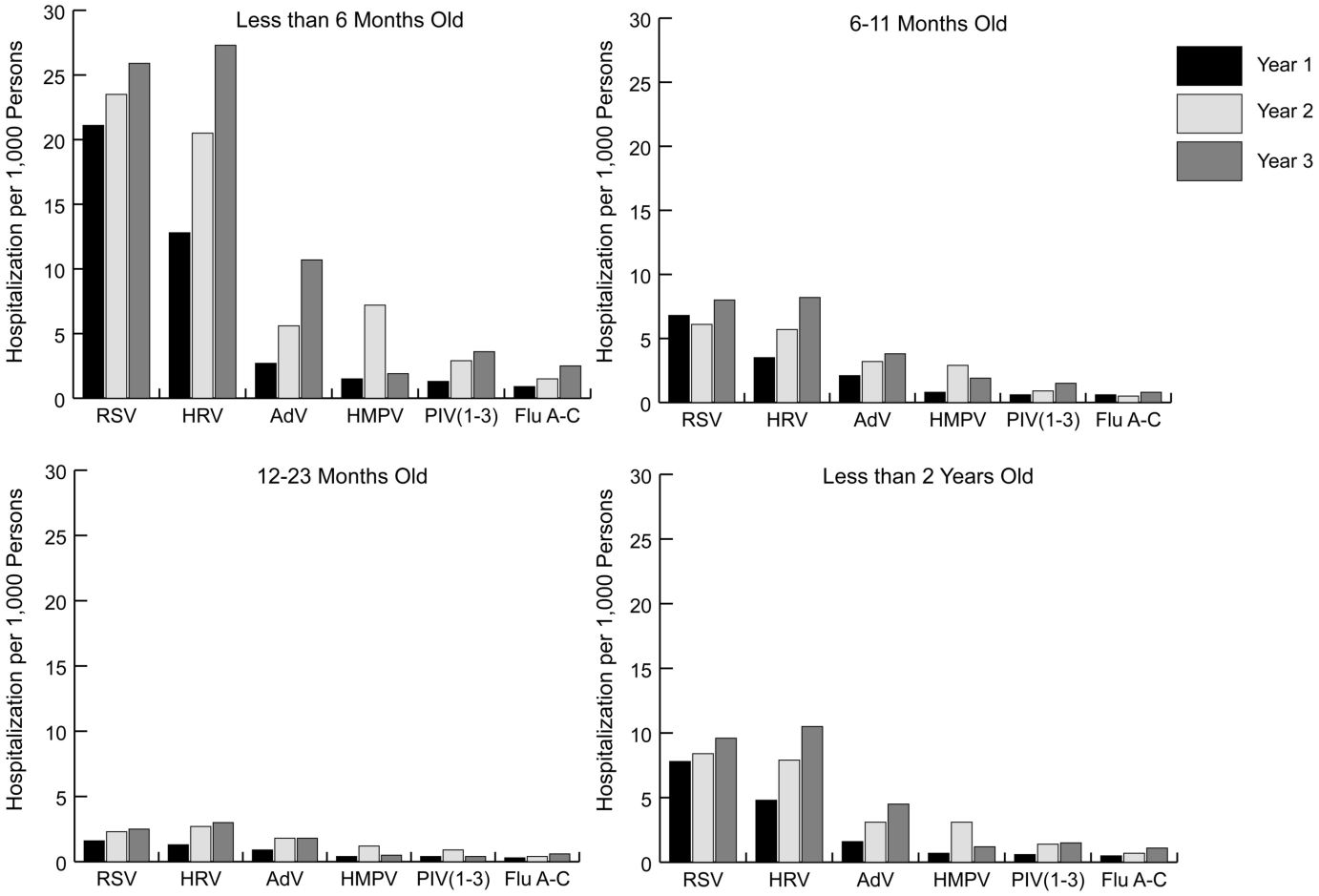

Figure 3 Hospitalisation rates by age group and years. AdV, adenovirus; HMPV, human metapneumovirus; HRV, human rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

7%, p<0.001) compared with virus-negative children. In contrast, virus-negative children were significantly more likely to be younger (table 1); present with fever (64% vs 54%, p<0.001), vomiting (23% vs 15%, p<0.001), diarrhoea (15% vs 9%, p<0.001), poor appetite (29% vs 20%, p<0.001) and seizures (7% vs 3%, p<0.001); have a diagnosis of suspected sepsis (50% vs 24%, p<0.001) and febrile seizure (5% vs 2%, p<0.001).

When comparing RSV only with other single detections, that is, HRV only, AdV only, HMPV only, PIV only and influenza only, children with RSV only were significantly younger than children with HMPV only (3.4 months vs 6.1 months, p<0.001) (table 1). RSV-only subjects were significantly more likely to require oxygen therapy compared with HRV-only (42% vs 29%, p<0.001), AdV-only (42% vs 21%, p<0.001) and PIV-only children (42% vs 23%, p<0.001) (table 1). Additionally, RSV-only children were significantly more likely to have lower vitamin D levels (15.3 ng/mL vs 18.5 ng/mL, p<0.001), receive antibiotics prior to hospitalisation (47% vs 32%, p<0.001) and less likely to have UMCs than HRV-only children (9% vs 15%, p<0.001) (table 1). RSV-only subjects were also significantly less likely to present with fever and more likely to present with a cough, shortness of breath and have flaring on examination compared with HRV-only, AdV-only, PIV-only and influenza-only children (online supplemental figure 1). RSV-only subjects significantly were less likely to have seizures/convulsions but more likely to have wheezing on examination compared with HRV-only and AdV-only children (online supplemental figure 1). RSV-only subjects significantly were less likely to present with diarrhoea but more likely to have cyanosis on examination compared with HRV-only, influenza-only and AdV-only children (online supplemental figure 1).

Risk factors for illness severity

Longer LOS was associated with younger age (adjusted OR (aOR) 0.47, 95% CI 0.38 to 0.59), lower gestational age (aOR 0.93, 95% CI 0.86 to 0.99), lower birth weight (aOR 0.85, 95% CI 0.75 to 0.96), lack of breast feeding (aOR 0.72, 95% CI 0.59 to 0.88), lower vitamin D level (aOR 0.86, 95% CI 0.76 to 0.98), UMC (aOR 1.71, 95% CI 1.37 to 2.13), virus detection (1.24, 95% CI 1.03 to 1.48) and diagnoses of pneumonia (aOR 2.07, 95% CI 1.65 to 2.60) or suspected sepsis (aOR 2.44, 95% CI 1.94 to 3.06) (table 2). Since early deaths would distort this observation, a sensitivity analysis excluding children who died was performed and still showed a significant association. Younger age (aOR 0.23, 95% CI 0.17 to 0.31), lower gestational age (0.90, 95% CI 0.82 to 0.98), lack of

Table 2 Demographic characteristics and clinical outcomes of study population

	Univariate				Multivariable		
	Risk of death				Length of stay		
	N	Death n=31 (%)	Alive n=3105 (%)	P values	Adjusted OR	P values	95% CI
Median age in months	3136	1.71 (1.23, 3.98)	3.5 (1.6, 8.5)	0.007	0.47	<0.01	0.38 to 0.59
Gestational age in weeks	3136	40 (37, 40)	40 (38, 40)	0.52	0.93	0.036	0.86 to 0.99
Birth weight	3134	2.80 (2.44, 3.38)	3.0 (2.5, 3.5)	0.25	0.85	0.012	0.75 to 0.96
Smoke exposure	3135	71%	77%	0.46	1.00	0.961	0.85 to 1.16
Sex male:female	3136	48%	60%	0.17	1.05	0.520	0.91 to 1.20
Breast feeding	3136	65%	84%	0.003	0.72	0.001	0.59 to 0.88
Vitamin D level	2661	20.4 (4.7, 25.0)	16.4 (5.2, 26.0)	0.75	0.86	0.021	0.76 to 0.98
UMC	3136	42%	11%	<0.01	1.71	<0.01	1.37 to 2.13
Virus positive	3136	68%	82%	0.049	1.24	0.022	1.03 to 1.48
Pneumonia	3136	32%	12%	<0.01	2.07	<0.01	1.65 to 2.60
Sepsis	3136	52%	29%	0.01	2.44	<0.01	1.94 to 3.06
Bronchiolitis	3136	3%	17%	0.04	0.80	0.056	0.63 to 1.01
Bronchopneumonia	3136	13%	32%	0.02	0.98	0.857	0.80 to 1.21
	Oxygen therapy						
	N	Oxygen n=1013 (%)	No oxygen n=2124 (%)	P values	Adjusted OR	P values	95% CI
Median age in months	3137	2.9 (1.4, 6.5)	4.0 (1.7, 9.2)	<0.01	0.23	<0.01	0.17 to 0.31
Gestational age in weeks	3137	40 (37, 40)	40 (38, 40)	<0.01	0.90	0.02	0.82 to 0.98
Birth weight	3135	3.0 (2.5, 3.4)	3.0 (2.6, 3.5)	0.03	0.96	0.63	0.81 to 1.13
Smoke exposure	3136	75%	77%	0.31	0.86	0.16	0.71 to 1.06
Sex—male	3137	58%	61%	0.51	0.83	0.048	0.70 to 0.998
Breast feeding	3137	82%	85%	0.01	0.67	0.005	0.40 to 0.94
Vitamin D level, ng/mL	2664	14.9 (4, 25)	17.3 (6, 26.4)	<0.01	0.82	0.02	0.70 to 0.97
UMC	3137	16%	10%	<0.01	1.95	<0.01	1.49 to 2.56
Virus positive	3137	87%	79%	<0.01	1.34	0.02	1.04 to 1.71
Pneumonia	3137	21%	9%	<0.01	2.94	<0.01	2.22 to 3.89
Sepsis	3137	20%	33%	<0.01	0.21	<0.01	0.15 to 0.27
Bronchiolitis	3137	21%	15%	<0.01	1.05	0.72	0.79 to 1.41
Bronchopneumonia	3137	28%	34%	<0.01	1.06	0.69	0.80 to 1.39
	ICU admission						
	N	ICU Stay n=284 (%)	No ICU Stay n=2856 (%)	P values	Adjusted OR	P values	95% CI
Median age in months	3140	2.1 (1.1, 6.1)	3.6 (1.7, 8.6)	<0.01	0.47	<0.01	0.30 to 0.74
Gestational age in weeks	3140	40 (37,40)	40 (38, 40)	0.017	0.93	0.32	0.81 to 1.07
Birth weight	3138	2.9 (2.44, 3.30)	3.0 (2.6, 3.5)	0.001	0.87	0.28	0.67 to 1.12
Smoke exposure	3139	74%	77%	0.35	0.89	0.46	0.65 to 1.22
Sex	3140	55%	61%	0.53	0.80	0.11	0.60 to 1.05
Breast feeding	3140	76%	85%	<0.01	0.63	0.01	0.44 to 0.91
Vitamin D level, ng/mL	2666	18.2 (5.3, 25.5)	16.4 (5.2, 26)	0.81	1.08	0.55	0.83 to 1.41
UMC	3140	23%	11%	<0.01	2.51	<0.01	1.71 to 3.68

Continued

Table 2 Continued

	ICU admission						
	N	ICU Stay n=284 (%)	No ICU Stay n=2856 (%)	P values	Adjusted OR	P values	95% CI
Virus positive	3140	77%	82%	0.036	0.84	0.31	0.59 to 1.18
Pneumonia	3140	28%	11%	<0.01	3.12	<0.01	2.16 to 4.50
Sepsis	3140	43%	28%	<0.01	1.14	0.53	0.76 to 1.71
Bronchiolitis	3140	11%	18%	<0.01	0.78	0.36	0.46 to 1.32
Bronchopneumonia	3140	19%	33%	<0.01	0.81	0.37	0.50 to 1.30
	MV						
	N	MV n=111 (%)	No MV n=3025 (%)	P values	Adjusted OR	P values	95% CI
Median age in months	3136	3.2 (1.4, 6.7)	3.5 (1.6, 8.5)	0.16	0.28	<0.01	0.15 to 0.53
Gestational age in weeks	3136	40 (37, 40)	40 (38, 40)	0.28	0.88	0.19	0.72 to 1.07
Birth weight	3134	3.0 (2.5, 3.3)	3.0 (2.5, 3.5)	0.18	0.998	0.99	0.69 to 1.44
Smoke exposure	3135	77%	77%	0.99	0.99	0.97	0.63 to 1.57
Gender	3136	59%	60%	0.70	0.89	0.56	0.60 to 1.32
Breast feeding	3136	86%	84%	0.48	1.53	0.17	0.84 to 2.81
Vitamin D level, ng/mL	2663	20.1 (5.7, 26.6)	16.4 (5.2, 25.9)	0.35	1.31	0.14	0·92 to 1·85
UMC	3136	20%	11%	<0.01	1.91	0.02	1.11 to 3.28
Virus positive	3136	80%	81%	0.75	0.88	0.62	0.52 to 1.48
Pneumonia	3136	23%	12%	<0.01	3.33	<0.01	1.85 to 6.0
Sepsis	3136	22%	29%	0.09	0.48	0.02	0.26 to 0.89
Bronchiolitis	3136	19%	17%	0.64	1.61	0.17	0.81 to 3.20
Bronchopneumonia	3136	33%	32%	0.78	2.03	0.03	1.05 to 3.90

ICU, intensive care unit; MV, mechanical vent; UMC, underlying medical condition.

breast feeding (0.67, 95% CI 0.40 to 0.94), lower vitamin D levels (0.82, 95% CI 0.70 to 0.97), UMC (1.95, 95% CI 1.49 to 2.56), virus detection (aOR 1.34, 95% CI 1.04 to 1.71), sex (aOR 0.83, 95% CI 0.70 to 0.99) and diagnosis of pneumonia (aOR 2.94, 95% CI 2.22 to 3.89) or sepsis (aOR 0.21, 95% CI 0.15 to 0.27) were associated with oxygen therapy (table 2). Diagnosis of pneumonia (3.12, 95% CI 2.16 to 4.50), younger age (0.47, 95% 0.30–0.74), UMC (aOR 2.51, 95% CI 1.71 to 3.68) and lack of breast feeding (aOR 0.63, 95% CI 0.44 to 0.91) were associated with an ICU stay (table 2). MV was associated with a diagnosis of pneumonia (aOR 3.33, 95% CI 1.85 to 6.0), or bronchopneumonia (aOR 2.03, 95% CI 1.05 to 3.90), younger age (0.28, aOR (0.15–0.53) and UMC (1.91, 95% CI 1.11 to 3.28), but was less likely with the diagnosis of suspected sepsis (0.48, 95% 0.26 to 0.89) (table 2). The number of deaths was too low to support a multivariable analysis. However, in a univariate model, younger age, lack of breast feeding, UMC, virus negativity and diagnoses of pneumonia or suspected sepsis were all associated with death, while bronchiolitis or bronchopneumonia was less likely to be associated with death (table 2).

DISCUSSION

Our study revealed that respiratory viruses are the predominant pathogens among young hospitalised children who present with fever and/or respiratory symptoms in Amman, Jordan. The most common diagnoses were bronchopneumonia, suspected sepsis, bronchiolitis, pneumonia and pertussis-like cough. Only one-fifth of the children enrolled had no virus detected. Consistently, during each of the three winter seasons, ~95% of children hospitalised during winter months tested positive for a virus, with RSV and HRV being the most common viruses identified. Both RSV and HRV rates were higher than expected compared with US-based studies.[18 23 24] Specifically, RSV rates were similar to rates from Turkey, Norway and Austria[25–29]; higher than the US and Netherlands[23 30]; and lower than UK, Spain and Denmark.[31–34] Rates of influenza, HMPV and PIV1–3 in our cohort were similar to US reports.[35–38] Our estimates of the burden of influenza, HRV, HMPV, PIV 1–3 and RSV fill a gap in knowledge from the Middle East.[7 8]

Hospitalised children who were virus negative were more likely to be younger, febrile and symptomatic with non-respiratory symptoms. Moreover, the overall

illness severity of virus-negative children was greater than those with a confirmed viral cause, with more frequent suspected sepsis, seizures, ICU admission and/or mortality. In contrast, virus-positive children were more likely to present with severe lower respiratory tract infection as indicated by the higher frequency of cough and shortness of breath, wheezing, flaring and cyanosis on examination, and increased oxygen therapy requirement. These clinical differences were dramatic and may serve as helpful clinical predictors of likely viral diagnosis prior to the availability of more definitive laboratory findings. In addition, 68% of the children who died had at least one respiratory virus detected, noting the association of severe disease with viral detection in these children. Since antibiotic use was nearly universal in children with positive detection of respiratory viruses, targeted interventions for antibiotic stewardship may also be warranted.

Since RSV was the most common virus detected, we compared the clinical presentations of RSV only to single detection of other respiratory viruses. RSV-only children were more likely to present with cough and shortness of breath; have flaring, wheezing and cyanosis on physical examination; and less likely to present with fever, diarrhoea and seizures compared with other respiratory viruses. Interestingly, the clinical presentations of RSV and HMPV were indistinguishable, other than age: HMPV-only children were older. A surveillance study of RSV-positive children in India found the presence of cough, fast breathing, crepitation and hypoxia to be independent predictors of RSV infection.[39] Durani *et al* found the combination of cough, wheezing and retractions to be good predictors of RSV infection.[39 40] Our study also found cough to be a strong predictor of the presence of virus, including RSV. In addition, the RSV-positive children in our study were more likely to require oxygen therapy, suggesting these children presented with more severe LRT disease. We also documented RSV being more severe than other viruses in a previous pilot study.[14] Therefore, if preventive measures such as vaccines become available for RSV, Jordanian children would benefit greatly.

We examined independent risk factors for several measures of illness severity: LOS, oxygen therapy, ICU admission, MV and death. Universally, younger age, UMC and the diagnosis of pneumonia were associated with all five illness severity markers. Lack of breast feeding was associated with all severity markers except for MV. Therefore, the promotion of breast feeding is an essential public health intervention for reducing the severity of respiratory illnesses. Our study found that lower vitamin D levels were associated with longer LOS and a higher probability of oxygen use. Two studies identified an association between lower infant vitamin D levels and increased risk of ARI, and one study found lower levels of vitamin D in children admitted into the ICU with bronchiolitis or pneumonia compared with those admitted to the wards.[41–43] Observational evidence supports further interventional studies to determine if vitamin D supplementation could reduce respiratory illness severity in this population.

A principal strength of our study was its basis of over three full years of surveillance data from a public hospital serving the poor and lower-middle class children of a large city in the Middle East North Africa (MENA) region. Prior to this study, very few large prospective studies of respiratory viral diseases of children have been completed in this setting. Our ongoing surveillance enabled us to test samples during the time of the first MERS-CoV outbreak in 2012, confirming that none of the children in this study were admitted with MERS-CoV.[44] Our state-of-the-art molecular diagnostics give one of the best assessments to date of respiratory viral aetiologies of hospitalised children in any MENA nation. Limitations included having only 5 days of surveillance per week, though we did weighted analysis in our population burden estimates to adjust for missing days. Also, we did not test for all respiratory viruses (eg, parainfluenza 4, non-MERS coronaviruses), so the viral burden is underestimated. Lastly, this study is limited to children from low-income and middle-income population seeking care at a single health facility in Amman, and thus is not generalisable to the entire population of Jordan.

The respiratory viral burden is likely to be substantial for the entire MENA region.[7] Prevention strategies such as breastfeeding promotion, vitamin D supplementation and future RSV vaccines could reduce regional ARI burden. Further studies that include groups representing the broader population in Jordan are needed.

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