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ORIGINAL ARTICLE

Respiratory virus detection and co-infection in children and adults in a large Australian hospital in 2009–2015

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Aim: This hospital network-based retrospective observational study aimed to describe the prevalence and seasonality of paediatric and adult viral respiratory pathogens and their rates of co-infections, following the introduction of a rapid multiplex molecular diagnostic assay.

Methods: All nasopharyngeal samples tested in patients presenting to Monash Health, Melbourne, Australia, from August 2009 to July 2015 by means of multiplex tandem polymerase chain reaction using the Respiratory Pathogen 12Plex kit (AusDiagnostics) were included in the analysis.

Results: There were 28 729 patient samples analysed after duplicate samples were excluded. Positive results were twice as likely in paediatrics, 7573/11 491 (65.9%), compared to adults, 5410/17 238 (31.4%). Co-infection was more frequent in paediatrics, 1642/7573 (21.7% of positives), compared to adults 299/5410 (5.5%). Adenovirus had a high prevalence as a co-infection, 639/990 (64.5%), in paediatrics. Testing frequency increased by 179% in the paediatric group and by 949% for adults over the 6 years of observation.

Conclusions: This study demonstrated a significant difference in the positive detection rate of pathogens and co-infections between the population groups. Adenovirus had a surprisingly high prevalence as a co-infection, especially in paediatric patients. Over the study period, rapid uptake of the test was observed, especially in adults. This raises concerns about how we can ensure that testing remains rational and is able to be provided in a cost-effective manner in the future.

Key words: co-infection; paediatric; polymerase chain reaction; respiratory; viral.

What is already known on this topic

1 Respiratory tract infections are the most common cause for hospital admission in young children.
2 Viral pathogens can account for up to 50% of community-acquired pneumonia in children.
3 Rapid molecular diagnostics allow for the easy detection of viral pathogens, including multiple pathogens.

What this paper adds

1 There is a high prevalence of adenovirus in co-infections in paediatric population.
2 Although low overall, there is also a high prevalence of adenovirus in co-infection amongst adults.
3 The uptake rate of rapid molecular testing over 6 years since its introduction has been small in paediatrics compared to in the adult cohort.

Respiratory tract infections (RTI) are the most common cause of hospitalisation in young children.[1,2] Viral pathogens can account for greater than 50% of community-acquired pneumonia (CAP) in children,[2,3] with viral aetiologies being attributed to up to 30% of adults admitted with CAP,[4] a condition with an overall mortality of 3%.[5] This is significant given that over 15–20% of severe CAP cases in adults have been attributed to influenza alone.[5,6]

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The increased availability of rapid molecular diagnostics for multiple respiratory viruses has allowed for the easier identification of viral pathogens.[7] The use of amplification during these diagnostic assays enables the detection of even very low levels of virus, providing a high sensitivity.[8,9] There has been a resultant rapid uptake of testing in recent years, largely replacing less sensitive and/or more labour-intensive culture and antigen detection methodologies. Given the emergence of improved therapies for viral respiratory disease, identification of the relative contribution of viruses to RTI presentations may aid in both clinical management and informing the health system of the prevalence of these pathogens.

We aimed to describe the frequency of viral respiratory pathogen detection in both paediatric and adult populations at our health network following the implementation of a respiratory

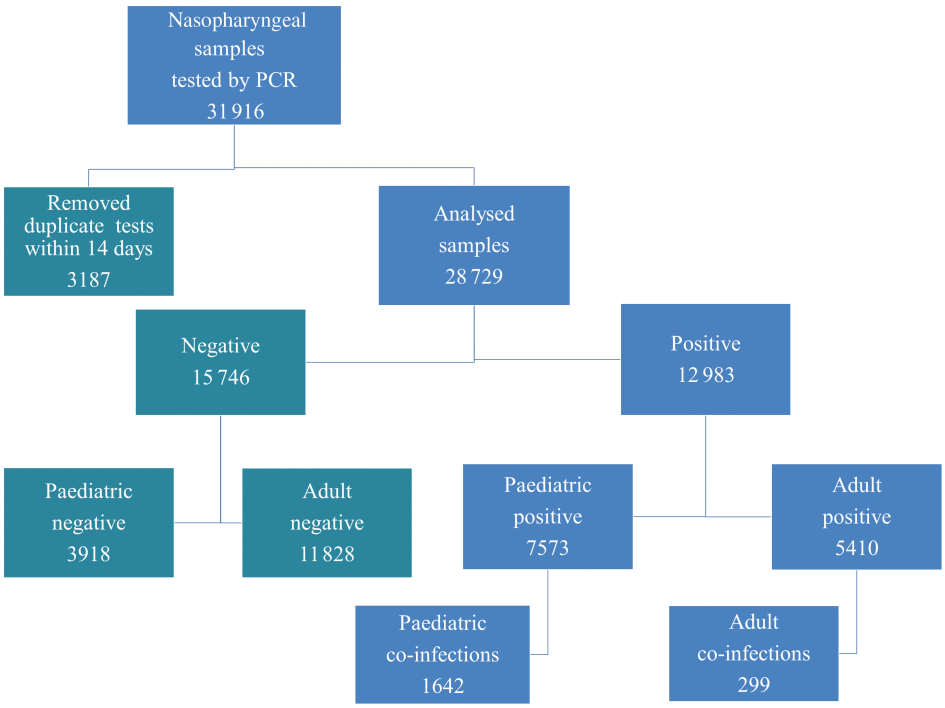

Fig. 1 Analysed respiratory multiplex polymerase chain reaction (PCR) samples obtained from Monash Health, Melbourne, Australia from August 2009 to July 2015.

multiplex polymerase chain reaction (PCR) assay. Analysis was conducted to describe the prevalence of co-infections as the improved sensitivity and increased use of multiplex analysis has led to the increased detection of more than one potential pathogen in a single sample.[10,11]

Methods

Setting

The study was conducted at Monash Health, Melbourne, Australia, a 2100 inpatient bed hospital regional network, incorporating Monash Children's Hospital and three emergency departments. This encompasses a 2312 km^2 catchment region in the southeast of Melbourne. Monash Health services a greater community of 1.3 million residents, covering approximately 24% of Victoria's population, with secondary and tertiary hospitals for both children and adults within the network. All patients who were tested using respiratory multiplex PCRs were included: paediatric was defined as younger than 18 years of age, and adult was defined as 18 years and over.

Table 1 Individual respiratory pathogen results for all samples, adult and paediatric data, at Monash Health, Melbourne, Australia, from August 2009 to July 2015

Respiratory pathogen	Paediatric, *n* (%)	Adult, *n* (%)
Influenza A	467 (4.1)	1214 (7.0)
Influenza B	263 (2.3)	430 (2.5)
Respiratory syncytial virus	2038 (17.7)	664 (3.9)
Picornavirus	4069 (35.4)	2203 (12.8)
Parainfluenza 1	134 (1.2)	57 (0.3)
Parainfluenza 2	85 (0.7)	52 (0.3)
Parainfluenza 3	555 (4.8)	294 (1.7)
Adenovirus	990 (8.6)	207 (1.2)
Human metapneumovirus	513 (4.5)	464 (2.7)
Bordetella pertussis	437 (3.8)	139 (0.8)
Any respiratory pathogen	7573 (65.9)	5410 (31.4)

Data collection

All respiratory multiplex PCR results were retrospectively extracted from the Monash Pathology laboratory information system spanning a 6-year period from August 2009 to July 2015. Only nasopharyngeal samples tested for all 10 respiratory pathogens on the multiplex panel were included in the study.

Laboratory testing

Nasopharyngeal samples were collected as aspirates or swabs by using mini-tipped flocked swabs containing a universal transport medium (Interpath, Melbourne, Australia). Total nucleic acid extraction was performed using the NucliSens easyMAG platform according to the instructions of the manufacturer (bioMerieux, Marcy l'Etoile, France), eluting 200 µL of sample into 50 µL of elution buffer, and then tested using multiplexed tandem PCR (MT-PCR) and a liquid-handling robotics system as previously described[12] (AusDiagnostics, Melbourne, Australia) using the Respiratory Pathogen 12Plex kit, which detects: influenza A (including H1N1 2009 influenza A), influenza B, respiratory syncytial virus (RSV), picornavirus (human rhinoviruses and human enteroviruses), parainfluenza 1, parainfluenza 2, parainfluenza 3, adenovirus (human types 1, 2, 3, 4, 5, 6, 7 and 8), human metapneumovirus and the bacteria *Bordetella pertussis*.

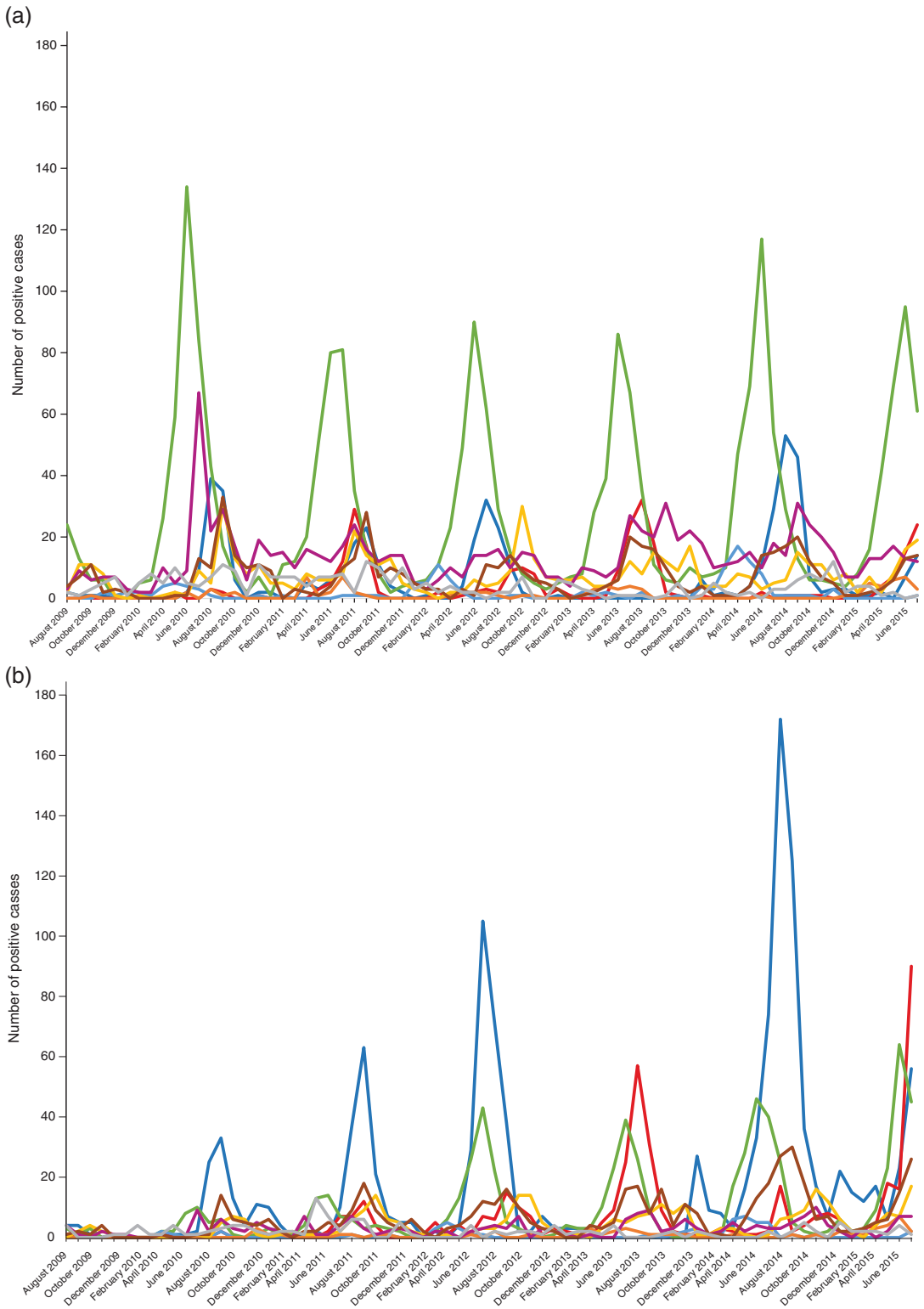

Fig. 2 (continues)

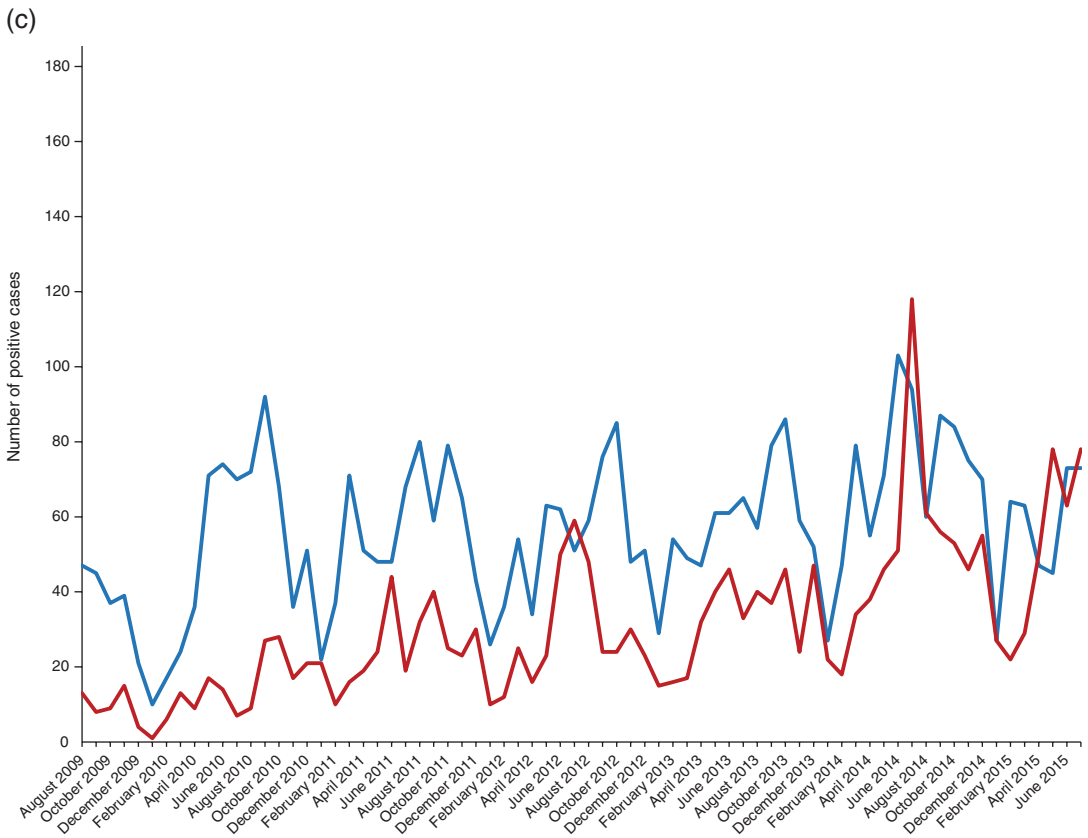

Fig. 2 Seasonality of detected respiratory pathogen: (a) Paediatric, (b) adult and (c) paediatric and adult picornavirus. (——), Influenza A; (——), influenza B; (——), respiratory syncytial virus; (——), parainfluenza 1; (——), parainfluenza 2; (——), parainfluenza 3; (——), adenovirus; (——), human metapneumovirus; (——), *Bordetella pertussis*. c: (——), Paediatric picornavirus; (——), adult picornavirus.

Definitions

The following inclusion and exclusion criteria were applied to the dataset to account for duplicate samples:

1 Any patient with a positive result and a repeat sample within 14 days that demonstrated the same result had the later results excluded for the main analysis.
2 If the repeat sample demonstrated the same viral pathogen as well as a new viral pathogen, then the repeat result was disregarded; however, the new positive result was included as a co-infection.
3 If the repeat sample within 14 days demonstrated a different pathogen from the initial sample, then both results were included as individual cases.
4 If multiple tests were performed within the same day, then a single result was included, and all others excluded; if both a positive and a negative result were detected from the same patient on the same day, then the positive sample was included.

For definition purposes, a positive sample designated the beginning of the infection period. Beyond a 14-day period, any repeat test was considered a new sample regardless of result. Single infection was defined as the detection of only one virus from a sample, and co-infection was defined as the detection of two or more pathogens from the single sample.

Data analysis and statistics

Descriptive analysis was performed using Microsoft Excel 2013 (Microsoft, Redmond, CA, USA). A two-sample test of proportions was performed using Stata 13 (StataCorp, College Station, TX, USA).

The number and percentage for each pathogen detected, and its co-infection, were determined. Detection rates for each pathogen and rates of co-infection were determined. A comparison between the paediatric and adult data for each pathogen was made, including pathogens more commonly isolated in each group.

Ethical approval

We state that the protocol for this quality assurance project was approved by the Monash Health Ethics Committee 11274Q.

Table 2 Co-infections with two respiratory pathogens only in paediatrics and adults presenting to Monash Health, Melbourne, Australia, from August 2009 to July 2015

	Paediatrics co-infections									
	Influenza A	Influenza B, *n*	RSV, *n*	Picornavirus, *n*	Parainfluenza 1, *n*	Parainfluenza 2, *n*	Parainfluenza 3, *n*	Adenovirus, *n*	HMPV, *n*	*Bordetella pertussis*, *n*
Influenza A	NA	0	21	36	0	0	6	19	11	6
Influenza B		NA	12	19	0	0	1	7	2	2
RSV			NA	400	4	1	20	91	22	18
Picornavirus				NA	30	16	108	319	92	95
Parainfluenza 1					NA	0	0	3	1	1
Parainfluenza 2						NA	1	5	1	0
Parainfluenza 3							NA	39	6	5
Adenovirus								NA	22	5
HMPV									NA	7
B. pertussis										NA
	Adult co-infections									
	Influenza A	Influenza B, *n*	RSV, *n*	Picornavirus, *n*	Parainfluenza 1, *n*	Parainfluenza 2, *n*	Parainfluenza 3, *n*	Adenovirus, *n*	HMPV, *n*	*B. pertussis*, *n*
Influenza A	NA	0	13	45	0	2	2	5	13	8
Influenza B		NA	9	8	3	1	1	4	4	1
RSV			NA	46	0	0	1	9	6	5
Picornavirus				NA	1	4	11	25	20	21
Parainfluenza 1					NA	0	0	0	0	0
Parainfluenza 2						NA	0	1	0	1
Parainfluenza 3							NA	3	3	5
Adenovirus								NA	1	2
HMPV									NA	0
B. pertussis										NA

Co-infections of three or more are not included in this data. HMPV, human metapneumovirus; NA, not applicable; RSV, respiratory syncytial virus.

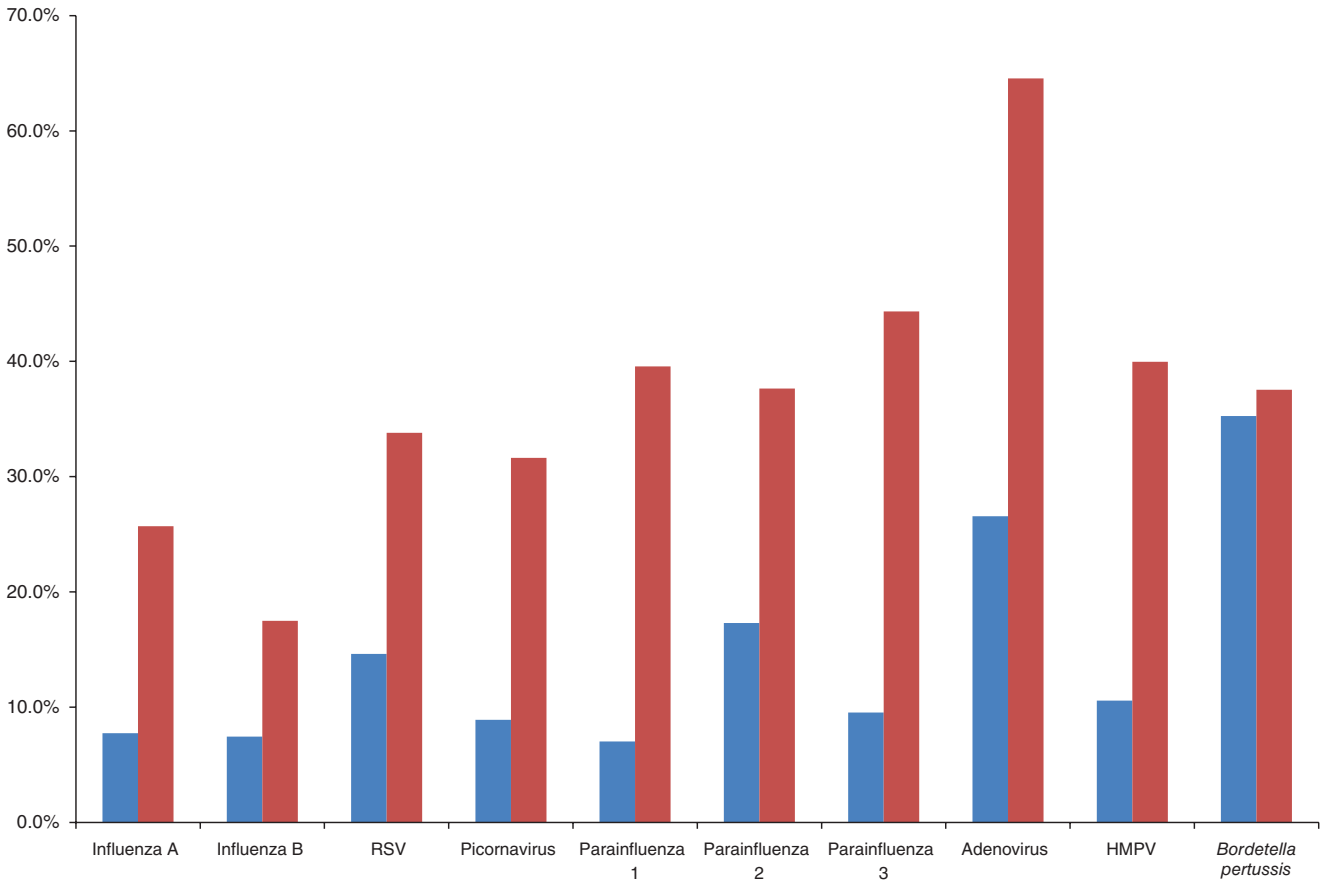

Fig. 3 Comparison of prevalence of respiratory pathogen detection as co-infection between paediatrics and adults. (——), Adults; (——), paediatrics. HMPV, human metapneumovirus; RSV, respiratory syncytial virus.

Informed consent

As this study was a retrospective extraction of non-identifiable data from the Monash Health pathology system, informed consent was not obtained from individual patients. The study was conducted with the Monash Health Human Research Ethics Committee approval, as mentioned above.

Table 3 Comparison of frequency of testing, and rate of positivity, per annum over the 6-year period from August 2009 to July 2015

Year	Paediatric, *n* (%)	Adult, *n* (%)
2009	1314 (69.1)	631 (33.0)
2010	1844 (68.7)	1491 (37.0)
2011	1836 (67.8)	2365 (37.8)
2012	1923 (63.1)	2673 (30.8)
2013	2222 (65.8)	4090 (28.5)
2014	2352 (62.7)	5988 (29.5)
Total	11 491 (65.9)	17 238 (31.4)

Each 12-month period referenced refers to August–July.

Results

A total of 28 729 patient samples were analysed following the removal of duplicate samples. Positive results (45.2%) were detected in 7573 paediatric samples (65.9%) and 5410 (31.4%) adult samples (Fig. 1). The most common pathogen detected was picornavirus, with the next most common pathogen being RSV in paediatrics and influenza A in adults (Table 1).

The seasonality of each pathogen is displayed for each group in Figure 2. Paediatrics showed a consistently high peak for RSV around June, while influenza A was the most prevalent in adults and was seen to peak around September. There was no seasonality noted for the picornavirus amongst either paediatrics or adults.

The detection of multiple respiratory pathogens was common as shown in Figure 3. There were 1454 paediatric and 284 adult samples that identified two pathogens; the most common combination for both groups was RSV and picornavirus. Amongst adults, this was closely followed by influenza A and picornavirus (Table 2). There were 179 paediatric samples with three pathogens (10.9%), yielding 35 different pathogen combinations. The most common three-pathogen combination was RSV, adenovirus and picornavirus. There were nine samples with four pathogens (0.5%) detected, with eight different pathogen combinations.

There were only 15 adult samples with three pathogens (5.0%), yielding nine different pathogen combinations. The majority of adenovirus detected in paediatrics was seen in the setting of other pathogens, 639 (64.5%), with adults also having a considerable proportion detected as a co-infection, 55 (26.6%).

There was an increase in the number of samples performed over the observation period. In the final year (August 2014–July 2015), there were 2352 paediatric samples and 5988 adult samples analysed, compared with only 1314 paediatric and 631 adult samples in the first year (Table 3).

Discussion

We report the results of a hospital network-based study comparing the detection of viral pathogens in children and adults, presenting from the same background population, using respiratory multiplex PCR assays. Paediatric samples were more than twice as likely to yield a positive result (65.9 vs. 31.4%).

A rapid uptake in the frequency of testing was observed across our study period, especially in the adult population, with an almost 10-fold increase in tests performed *per annum* when comparing across the 6 years. In children, a 79% increase was observed. Indications for testing include the ability to change patient management (either directly or for infection control reasons) or assisting communicable disease epidemiology. Respiratory testing is an uncomfortable procedure that incurs expense. The rapid uptake of testing over this period highlights the need for testing behaviours to remain rational and cost-effective.[13] In the setting of prolonged viral shedding long after clinical resolution of disease, a positive result may even disadvantage patients and health-care providers due to unnecessary periods of respiratory virus isolation in infected inpatients. The ability to perform a test and obtain a positive result alone is insufficient reason to conduct respiratory testing.

The proportion of paediatric PCR-positive respiratory samples is comparable with other studies with reported rates between 42.7 and 74%.[2,14–16] In the adult population, there is less available evidence for comparison that is not exclusive to specific subpopulations or inclusive of paediatric patients. From available data, our proportion of positive samples (31.4%) appears to be comparable.[17–19] Both the annual (17.7%) and seasonal (30.7%; May to August inclusive) detection rates of paediatric RSV infection are similar to other studies, with reported rates of 17.2–67.1%.[2,15,20–22] The detection rate of adult influenza A infection (annual 7.0%; seasonal 9.0%) is consistent with other studies.[17,23] RSV had a higher rate amongst the paediatric population compared with adults, whereas influenza A was more likely to be detected amongst the adult population, a previously recognised observation.[17]

The most commonly identified pathogen in both groups was the picornavirus (48.3% of total positive results, 31.3% paediatrics and 17.0% adults), making it easily the most commonly identified pathogen in both population groups. Across numerous studies, the picornavirus remains the most common respiratory pathogen identified.[3,24,25] Its presence also contributes to a significant proportion of co-infections overall and nearly all co-infections that contain three-pathogen combinations. This raises the question of a possible role for the picornavirus in respiratory illness presentations in children and adults. This may be as a primary pathogen, a co-factor for other viral or bacterial infections or as a trigger for non-infective respiratory presentations such as asthma. It is important to recognise, however, that it is the high detection of the picornavirus amongst healthy individuals has been well described.[26] However, recent concomitant blood and respiratory PCR detection of the rhinovirus in children with signs of lower RTI suggests it may play a role in up to one in six children with RTI who test positive for the rhinovirus.[27]

The incidence of co-infections in paediatric positive results (21.7%) is marginally higher than described in other studies (11.3–20.6%),[2,22] with previous studies also demonstrating lower co-infection rates in adults (5.5%).[28] The high proportion of adenovirus infections presenting as a co-infection has also been recently described amongst children with CAP.[29] Although not described on this scale previously, the adenovirus is also present in a significant proportion of adult co-infection.

Recently, there has been a large-scale prospective study from southern China describing respiratory viral infections in children and adults, usually through PCR techniques.[30] There was a different selection of viruses tested: influenza (A, B, C), RSV, adenovirus, human metapneumovirus, parainfluenza virus, human coronavirus and human bocavirus. Their rate of overall positive result (39.2%) was lower. Our identified rate of co-infection was significantly higher (6.6 vs. 3.4% of all samples and 15.0 vs. 9.6% of positive samples), which may be explained by the differences in viruses tested, population factors or indications for testing.

Our multiplex PCR tested for 10 common respiratory pathogens and therefore introduced several limitations. We were unable to determine the individual contribution of enterovirus or rhinovirus as they were both reported as picornavirus based on the same 5′ target. There are many other respiratory viral pathogens that we did not test for (e.g. coronavirus, bocavirus, influenza C). Inclusion of these may help close the 'diagnostic gap' in 34.1% of paediatric and 68.6% of adult tests that were negative. Our study demonstrates relative reproducibility in seasonality in respiratory pathogens amongst those requiring presentation or admission to hospital. In the absence of correlating clinical data, it is not possible to conclude the presence of a respiratory pathogen as causative for clinical disease.

The high sensitivity of molecular diagnostic assays and their ability to detect low levels of virus have led to a high yield for testing, with up to 95% of children presenting with RTI undergoing respiratory viral pathogen detection.[9] However, the interpretation of positive results is more complex. It may represent the causative pathogen for the presenting illness, prolonged shedding from a past infection or simply an asymptomatic infection. Since the introduction of this testing, asymptomatic carriage of respiratory pathogens on confirmed testing has been described at between 40 and 68%.[9,31,32]

The high prevalence of the picornavirus in co-infections has been described previously[23]; however, as far as we know, this is the first time the high prevalence of the adenovirus has been documented. It does, however, raise the question regarding the clinical significance and burden of disease associated with those presenting with single-pathogen detection versus co-infection. With the advent of increasing respiratory viral therapeutics and vaccines in pre-clinical and clinical development, our ability to understand the potential roles of many of

these viruses is likely to deepen, from both clinical trials and post-licensure data.

Conclusions

The ability of sensitive molecular assays has improved our ability to provide a potential explanation for their symptoms. However, old challenges remain and the high sensitivity of these tests raises new issues. The old challenge of whether a positive test regularly changes the clinical management of the patient tested remains under debate. The ability of the newer assays to detect non-viable viral RNA or DNA adds to the complexity of interpreting positive results, and may help explain some of the co-infections. Nevertheless, they continue to improve our ability to understand not only infectious diseases epidemiology but also the complex interaction of potential respiratory pathogens with adult and child hosts.

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