Viral etiology and the impact of co-detection in young children presenting with influenza-like illness

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Keywords:	Respiratory infection, Viral infection, Child, Co-detection

Please note: Reviewer comments are italics with authors' response in standard font. Corresponding page numbers are applicable to the revised manuscript with all track changed accepted.

Reviewer 1 comments

Abstract
Suggest clarify that the study was restricted to influenza seasons, and describe the setting were children were seen and samples collected.
Response
Information on the restrictions on enrolment, study setting and samples collected were amended in the abstract (p.5).

Please double check calculations as some appear to be a little off – this may be due to some rounding or observations with missing values but this is not clear in the results section (see additional comments below).
Response
As the reviewer has suggested, the differences in the presented results in the Abstract were due to rounding. To minimise confusion, we have amended the abstract to report results up to 1 decimal place as appropriate (pp.5-6).

The calculation of the predicted probabilities from the logistic regression model needs to be clearly described in the methods section. Whether the predicted probability for flu + RSV is significantly higher than others need to be clarified as well.
Response
We have clarified this in the abstract of the revised manuscript (pp.5-6).

Introduction
Suggest reconcile description of study settings with information from methods, as apparently not all recruitments were done at the emergency department
Response
To simplify analyses and minimise confusion regarding the study setting, we have chosen to exclude children enrolled from general practises in the revised manuscript. Only 131 were recruited from general practice in 2008-2009 before this arm of the study was stopped. All children included in the analyses were enrolled while they were transiting through the emergency department. A portion of these children would subsequently have been admitted as inpatients. We have included additional information on the timing and location of enrolment in the Introduction (p.7) with further information included in the Methods section (p.8). The number of patients excluded from general practice are included in the Results section (p.12).

Methods
Settings and participants
Suggest clarify how the influenza seasons were defined, based on calendar week/month?, laboratory surveillance?, etc.
Response
The start and end of influenza seasons were defined by the Infectious Diseases Surveillance Unit at PathWest Laboratory Medicine WA using a combination of indicators, including weekly proportion of laboratory influenza tests positive. As a guide, two consecutive weeks with over 10% influenza test positive often coincides with the beginning of influenza season in WA. The manuscript has been amended to include additional information on how influenza seasons were defined (p.8).

Clarify the different settings that were used for enrolment by season, and describe the distribution of these patients in results (e.g. % of children enrolled from inpatient facilities, etc.). How were hospitalized children treated in the analysis, for example in the assessment of risk of hospitalization?

Response

We acknowledge the confusion in the distribution of enrolment settings and consequently have opted to exclude data from all children enrolled at general practises (see response to Introduction comments above). All children presented to the emergency department of a single hospital and were either admitted or discharged home. Hospital admission was a key outcome of interest.

How was influenza vaccination data collected? What proportion of self-report was verified? What proportion of children were fully vaccinated?

Response

Vaccination data were collected through parental-report and confirmed through the Australian Childhood Immunisation Register. If there was ongoing uncertainty, immunisation providers were contacted. Data on vaccination status (apart from influenza vaccination) were not collected. The manuscript has been amended to include this information (p.9).

Was antiviral use before sample collection measured or accounted for in the analyses?

Response

Only data on antiviral use post-enrolment were collected as part of the follow-up questionnaire. However, only 9 patients were prescribed anti-viral medications and as such, data on anti-viral medications were not included in the analyses.

Respiratory virus detection

Several tests are described for different viruses but it is unclear if testing was systematic throughout the study period or some changes in testing were implemented over time. This seems very crucial as not all the described tests may have similar performance for viral detections. This information needs to be clearly described in the methods section.

Response

Testing was consistent throughout the study with all patients undergoing the same panel of tests with the only exception being testing for human metapneumovirus (hMPV). References detailing the PCR assays designed and validated specifically for this study have been included in the manuscript (reference #14-16). Testing for hMPV was based on clinical need. In addition, during the lifetime of the study, a PCR assay for hMPV was designed and implemented, gradually replacing the previously utilised antigen detection assay. Although infrequently detected, we have elected to include samples using both hMPV assays given the importance of this pathogen. This information has been included in the Methods section (p.10) and we have also acknowledged the limitations of using two assays in the Discussion section (p. 16).

Also, please clarify if all samples were tested for all study viruses – this is important to confirm that samples had equal chance of having co-detections identified.

Response

All samples were tested for all study viruses (see response above).

Was influenza and parainfluenza combined for all analyses? Suggest clarify the rationale for this decision – if this is the case then the abstract, discussion and conclusions need to be revised accordingly.

Response

Influenza virus subtypes (i.e. Influenza A, B and C) were grouped together. Likewise, subtypes of parainfluenza (i.e. parainfluenza types 1-4) were grouped together. Influenza and parainfluenza were treated as separate groups. This point has been clarified in the Methods section (p. 10).

Definitions and statistical analysis
Clarify how the children enrolled in the inpatient setting were considered for the study of hospitalization as an outcome.
Response
As all children transited through the emergency department, all children were treated in the same manner in the regression model with hospitalisation as the outcome measure.

How was age (covariate) accounted for in the regression models? The table shows data as a categorical variable but use of age as a continuous variable is warranted to minimize residual confounding.
Response
In the original manuscript, age was included in the model as a categorical variable with 5 subgroups (i.e. 6-11 months, 12-23 months, 2 years, 3 years and 4 years). We have since re-assessed models that include age as a continuous variable and found no difference in the model fit whether age was used as a continuous or categorical variable. As such, we have chosen to retain age as a categorical variable for the easier interpretation of the results. We have amended the manuscript to explicitly state this in Method sections (p.11) and as footnotes in Table 2.

Suggest clarify the models were used to calculate 'predicted' probabilities – it is important to provide details about model building strategies, as the estimated probabilities depend heavily on the model structure. Suggest consider influenza vaccination history before enrolment, antiviral use, duration of disease (from onset to testing), enrolment setting and respiratory season as additional covariates for the regression models. If additional information is available about presence of young children at home, that would be another covariate of interest.
Response
We acknowledge that the addition of the suggested covariates would help to strengthen the models. We have attempted to include these additional covariates into the models but found that this greatly reduces the number of cases contributing data to the model due to missing data. Comparisons, using the likelihood ratio test of standardised sample size, of the logistic regression model of hospitalisation with all the additional covariates requested showed no significant differences in model fit compared to the restricted model with only the covariates listed in the original manuscript. Inclusion of all requested covariates also resulted in quasi-separation of the data, which limited our ability to perform the post-hoc analyses used to produce Figures 2 and 3.

As such, we have decided to retain the covariates used in the original model. We have also included additional information on the model building strategies used in the Methods section of the revised manuscript (p.11).

Results

How many seasons were included in the study? Was the pandemic year distinct enough, so that it may warrant a sensitivity analysis excluding it from the main analysis?

Response

Influenza seasons from 2008 to 2012 (i.e. total of 5 influenza seasons) were included in this analyses. Given the questionable impact of co-detection is relevant in both pandemic and seasonal influenza seasons, we have kept the 2009 season in the analysis. While we acknowledge that it would be interesting to perform a sensitivity analyses excluding data from the pandemic year (2009), we feel that this would be outside the scope of this study. Furthermore, excluding data from this year would likely limit our ability to assess the effects of specific virus pairs given the limited sample size.

Clarify what proportion of children had comorbidities.

Response

The manuscript has been amended to include this information (p.11).

Suggest clarify whether the described antibiotic use refers to the enrollment event or the follow-up questionnaire?

Response

Data on antibiotic use was collected in the follow-up questionnaire. We have amended the manuscript to explicitly state that it refers to antibiotic use post-enrolment (p.12).

How many children had completed the follow-up questionnaire? It seems that the outcomes would be only known for those who completed it but cannot tell from the description. The proportion described with antibiotic data (52.6%) is very different from the proportion with hospitalization data (99%). The methods indicate that medical records review were done for hospitalizations but it is unclear whether antibiotic information was reviewed as well. This is very important as this potentially modify the n for some of the analyses.

Response

Of 2356 patients, 52.8% (n=1244) completed questions relating to outcomes (e.g. antibiotics use). Information on antibiotics data were not reviewed beyond data collected via questionnaire as the majority of patients were discharged from the emergency department and therefore, may not have adequate information recorded on subsequent antibiotic use. This information has been included in the Methods (p.9) and Results sections (p.12) of the amended manuscript.

Please double check the odds ratio calculations, for example in table 2 it seems that the crude odds ratio for cough should be 1.8? Currently shown as 2.01 – similar concern for next row [rhinorrhea], this might be a rounding issue but please verify. If there were children missing information in some of the variables listed in the table, it would be useful to clarify that as well. Suggest add variables included for adjustment in a footnote for Table 2.

Response

The discrepancies were due to cases with missing data counted as not having a particular symptom or outcome, as the reviewer suggested. To clarify this, we have included the number of children with missing data for each of the symptoms and outcomes as a note in Table 2. The variables included in the adjusted models have been noted in the footnotes. Please note that the values presented in the revised manuscript may differ from the original manuscript due to the exclusion of children recruited from general practises.

Although predicted probabilities are shown in figures 2 and 3, these come from the regression models and it is unclear whether the described comparisons indicated significant differences or whether chance could not be ruled out – this needs to be clarified carefully, and description of results and discussion revised accordingly, if necessary.

Response

We have amended the Methods (p.11), Results (pp.13-14) and Discussion sections (p.14) concerning these results to clarify this point. Figures 2 and 3 also include 95% confidence intervals to minimise the risk of over-interpretation.

Besides the described symptoms, it would helpful to show the distribution of actual medical diagnoses that these children received. For example were co-detections more often seen in children with diagnosis of otitis media or pneumonia? I think this is a very important part and needs to be added to the report.

Response

We acknowledge that discharge diagnosis would have added to the analyses presented in this manuscript. Unfortunately, data on the diagnoses received at discharge were not collected for this study. This point has been added as a potential limitation of this study in the Discussion section of the manuscript (p. 16).

Also, children may present with more than 1 symptom, but it is not clear how those were treated in the analyses, please clarify.

Response

We have interpreted this comment to mean that the reviewer is concerned about counting the same person contributing more than 1 data point in a model (i.e. if they had more than 1 symptom). As each symptom and outcome was treated as a separate model, the same child could present with more than 1 symptoms but will only be counted once in a particular model.

Discussion

Suggest clarify if described predicted probabilities were significantly higher than other groups to support statement in first paragraph.

Response

The predicted probability of hospitalisation for those with influenza and RSV co-detections were higher compared to those with influenza virus infection only with a trend observed compared with RSV virus infection only. To avoid confusion, we have clarified this point in the first paragraph of the Discussion section (p.14).

The description of the post-hoc power calculation is very confusing, what is the difference of interest for the calculation? Since the sample size is already fixed (this is a retrospective assessment), I am not sure how useful a power estimate is – the lack of precision can be appreciated directly by the width of the estimated confidence intervals. Suggest delete the power calculation description.

Response

The power calculations were provided to suggest the numbers required to detect differences between influenza and rhinovirus should a reader wish to do so in a future study. We acknowledge that this can be confusing and have deleted this description from the Discussion.

MPV detections were the lowest of all detections. How does the MPV % detections compare to other studies? Please discuss potential reasons for discrepancies.

Response

hMPV detections in this cohort was 1.0% (26/2487), which is lower than those shown in other studies (approximately 5-13% for this age group). This could be partly due to lower sensitivity of

immunofluorescence in comparison to PCR when detecting hMPV. This information has been included in the Discussion section of the revised manuscript (p.16).

Reviewer 2 comments

In general, I would dissuade the frequent use of "co-infection" and favor "co-detection," since this is largely a description of molecular detection of viruses and many of the positives (such as for rhinoviruses, in particular) may represent detection of viruses that reflect prolonged shedding in the nasopharynx but not current active infection. "Coinfection" is defined in the introduction, but I would still recommend that the authors use "co-detection" more frequently when describing results and conclusions.

Response

We acknowledge that we cannot distinguish between active infection and viral shedding and have changed the term "coinfection" to "co-detection" in the manuscript. We would refrain from using both terms within the same manuscript so as to not confuse the reader. We have also added further comments acknowledging the role of viral shedding in the Discussion section of the manuscript (p.16).

The abstract notes that rhinovirus (40%), influenza (29%), and RSV (27%) were the most commonly detected viruses. Please also present these percentages in the Results section and be clear that these are out of the 1728 with a virus detected (not of the 2487 eligible patients).

Response

Both the abstract (p.5) and Results section (p.16) has been amended to include this information.

The abstract notes that nasal swabs were tested, but the Methods section says a nasopharyngeal swab or aspirate was collected at enrollment. Please be specific as to what was routinely collected – was it nasal swab or aspirate? Was specimen type collected at clinician discretion? Were the swabs anterior nasal, mid-turbinate, or nasopharyngeal?

Response

Bilateral mid-turbinate nasal swabs (Copan Diagnostics, Murrieta, CA) placed into viral transport was the preferred specimen however if a nasopharyngeal aspirate (NPA) had already been performed, this sample was used rather than repeating the diagnostic test. 84.9% of samples collected were nasal swabs with the remainder NPA. This information has been added in the Methods section of the revised manuscript (p.9).

How much was added with the use of viral culture? I am not sure if there is room for this information to be included, but it would be interesting to know if testing by culture added to the virus detections. It is also worth noting that the number of hMPV positives seems very low relative to the other viruses – which causes one to question the sensitivity of the multiplex PCR and immunofluorescent assay for detection of hMPV. Please comment in the manuscript on this.

Response

Of 1630 patients with viruses detected, 34 patients (2.1%) had a virus detected only through culture. We have not included this information in the manuscript as it is not central to the main aims of this study.

We acknowledge that the proportion of children testing positive for hMPV in this cohort were lower than that in other studies and have included it as a point of Discussion (p.16) in the revised manuscript.

Line 55-56 on page 11 to top of page 12: Would note that children with co-detection of viruses were significantly younger compared with children with single virus detection; and while it is true that the percentage in out-of-home care was highest in the co-detection group, it was not statistically higher (as the confidence intervals overlap).

Response

We have amended the manuscript clarify these points in the Results (p.13).

Was there any information available regarding use of antivirals for treatment of influenza? It would be informative to include this information in the results, if available, and if there is sufficient space.

Response

Although data on antiviral use were collected from the follow-up questionnaire, we were unable to assess the role of antivirals in this study as only 9 patients were prescribed them (data not shown).

Because this was a study which required ILI for enrollment, all patients had fever as part of the case definition. This is well noted in the Discussion as a limitation, but would also recommend clarification in the Conclusions of the Abstract and throughout the Discussion that "Overall, coinfection has limited impact on clinical severity among young children with ILI." This should be clearer, for example, in the first and last paragraphs of the Discussion.

Response

We acknowledge that this is a valid point and have amended the abstract (p.6) and Discussion section (p.14, 16) in the revised manuscript to explicitly state this.

Title

Viral etiology and the impact of co-detection in young children presenting with influenza-like illness

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Keywords

Respiratory infection, Viral infection, Co-detection, Child

Running title

Viral etiology and impact of co-detection

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Key points

- Children frequently had multiple respiratory viruses detected
- Although common, children with multiple viruses more frequently had cough and rhinorrhoea
- Children with influenza and respiratory syncytial virus were most frequently hospitalised
- Routine screening and cohorting recommended only for common respiratory pathogens

Author contributions

CCB, PCR, PVE and DWS conducted the WAIVE study. PCR, DWS and CCB conceptualised this study. AL and ST conducted the laboratory work. ZVW and NTC conducted the preliminary data cleaning and analyses. FJL conducted data cleaning and analyses with assistance from HCM, NdK and CCB. FJL and ZVW jointly wrote the first draft of the manuscript. All authors have critically revised and approved of the final version of this manuscript.

Word counts

Abstract – 248 words

Manuscript – Approx. 3000 words

Abstract

Background

Children with acute respiratory tract infection (ARTI) frequently exhibit viral-viral co-detection, yet its clinical significance remains contentious. Using data from a prospective cohort of children with influenza-like illness, we described the virology of ARTI and determined the clinical impact of viral-viral co-detection.

Methods

Children aged 6-59 months presenting to a tertiary paediatric hospital with fever and acute respiratory symptoms were enrolled and nasal samples collected during influenza seasons in 2008-2012. Respiratory viruses were identified by culture and PCR. We compared demographics, presenting symptoms and clinical outcomes of children with single viral infection and viral-viral co-detection. We used logistic regression models and estimated marginal means to calculate the adjusted odds ratio and probabilities of symptom presentation, antibiotic prescription or hospitalisation.

Results

1630 of 2356 children (69.2%) had a virus detected, among whom rhinovirus (40.8%), influenza (29.5%) and respiratory syncytial virus (RSV; 26.4%) were most commonly detected. 24% of these had two or more viruses detected. After adjusting for demographic factors, children with co-detection had greater odds of presenting with cough (aOR=1.9, 95% CI:1.2-3.1), rhinorrhoea (aOR=1.8, 95% CI:1.1-2.9) than those with single infection, although both symptoms were common. Children with influenza and RSV combined had the highest probability of hospitalisation

(probability=55%, 95%CI:35-73%), significantly greater than those with influenza infection alone (probability=22%, 95%CI:16-29%).

Conclusions

Overall, co-detection has limited impact on clinical severity among children with influenza-like illness. However, specific pathogen pairs may be associated with more severe outcomes. Routine diagnostics to identify viral co-detection should be restricted to common pathogens.

1 Introduction

Acute respiratory tract infections (ARTI) in children place a significant burden on families and the community. Commonly recognized respiratory viral pathogens include influenza viruses, respiratory syncytial viruses (RSV), parainfluenza viruses, human rhinoviruses, adenoviruses and coronaviruses [1,2]. Advances in laboratory diagnostic techniques have resulted in the discovery of new viruses, including human metapneumovirus (hMPV) and polyomaviruses [3,4], yet a number of these pathogens have uncertain pathogenicity [5,6].

Co-detection can be defined as detection of two or more pathogens in a single sample. With the improved sensitivity, availability and affordability of modern diagnostics, viral-viral co-detections are being increasingly identified. The incidence of viral-viral co-detection has been reported between 15-45%, depending on age, location and testing methods [7–9]. The clinical significance of co-detection in ARTI remains contentious with the literature ranging from negligible to deleterious effects [9,10].

This study describes the virology of ARTI in children aged six months to four years who presented with influenza-like illness during influenza season to a tertiary paediatric hospital in Australia. This study also enabled us to specifically examine the impact of viral-viral co-detection on clinical symptoms and outcomes.

2 Materials and Methods

2.1 Study setting and patients

Western Australia (WA) spans 2.5 million square kilometres with a population of approximately 2.5 million people, 7% of whom are under 5 years of age [11]. Princess Margaret Hospital for Children (PMH) is the only tertiary paediatric hospital in the state and is located in metropolitan Perth where approximately 80% of the population resides [12].

Commencing in 2008, the Western Australia Influenza Vaccine Effectiveness (WAIVE) Study was an observational cohort study established to determine the effectiveness of inactivated influenza vaccine. Patient recruitment was conducted at PMH (and at selected general practises in metropolitan WA in 2008-2009). Due to small numbers recruited and differences in presentation, data from children presenting to general practises were removed from these analyses.

Patient recruitment coincided with the annual influenza seasons. The start and end of influenza seasons were defined by the Infectious Diseases Surveillance Unit at PathWest Laboratory Medicine WA using a combination of indicators, including weekly proportion of laboratory influenza tests positive. As a guide, two consecutive weeks with over 10% influenza test positive often coincides with the beginning of influenza season in WA. Further details on study design are described elsewhere [13].

All children 6-59 months of age presenting to PMH with a history of fever (by parental report) or with a measured temperature of greater than 37.5°C at

presentation, accompanied by at least one acute respiratory symptoms within the previous 96 hours, were eligible for enrolment. All children transited through PMH emergency department. A portion of these children were subsequently admitted to hospital with the remainder discharged home from the emergency department. Children with a known immunodeficiency disorder, current or recent immunosuppressive treatment, or who received immunoglobulin in the previous three months were excluded from the study.

Patient demographics, medical history and presenting symptoms were collected by parental questionnaire. Comorbidities recorded included prematurity, asthma, chronic cardiac, neurological or respiratory conditions. Influenza vaccination status was obtained by parental report and confirmed through the Australian Childhood Immunisation Register or by contacting immunisation providers. Vaccination status for other vaccines were not collected. A follow-up questionnaire of illness outcomes, including details of hospital admission(s), use of antibiotics and time to recovery, was provided to families to complete within 7-10 days after enrolment. A retrospective review of medical records was undertaken when hospitalisation data were incorrectly recorded or missing. No follow-up was conducted for antibiotics use if data were missing.

2.2 Respiratory virus detection

Children had bilateral mid-turbinate nasal swabs collected at enrolment (Copan Diagnostics Inc., Murrieta, CA). If a nasopharyngeal aspirate had already been collected by hospital staff as part of clinical care, this sample was used in lieu of a nasal swab. Viral culture (Madin-Darby Canine Kidney cells, Diploid lung fibroblasts)

and multiplex tandem PCR was used to detect all viruses except picornaviruses and hMPV [14,15]. Picornaviruses were detected using nested PCR [16] targeting the 5'UTR of the picornavirus genome with sequencing used to assist with identification of rhinoviruses and enteroviruses. hMPV was tested using an immunofluorescent assay (Simulfluor hMPV Immunofluoresent Assay; Millipore, Temecula, CA) and PCR. All patients were subjected to the same panel of tests and testing methods were consistent throughout the study period with the exception of testing for hMPV; testing for hMPV was based on clinical need. While both immunofluorescence and PCR assays were used throughout the study period, PCR testing was more common in later years.

For all viruses (except hMPV), positive viral detection was defined as detection by viral culture and/or PCR. Positive detection of hMPV was defined as detection by immunofluorescence and/or PCR. All influenza types/subtypes (i.e. influenza A/H1N1, A/H3N2 and B) were grouped for analysis. Similarly, subgroups of parainfluenza viruses (i.e. parainfluenza types 1-4) were grouped together for analysis. Infection was defined as detection of one or more of rhinovirus, influenza, RSV, parainfluenza, adenovirus, coronavirus or hMPV. Co-detection was defined as detection of two or more viruses in a single diagnostic sample.

2.3 Definitions and statistical analysis

Prematurity was defined as less than 37 weeks of gestation at birth. Out-of-home care was defined as attendance at playgroup, mothers' group, day-care centre, kindergarten or preschool. Length of stay in hospital refers to the duration from admission to discharge date. Symptoms investigated included cough, rhinorrhoea,

wheeze, dyspnoea, rash, diarrhoea and vomiting while outcomes investigated were antibiotic prescription and hospital admission.

Data cleaning and analyses were performed in Microsoft Excel, EpiBasic [17] and SPSS version 23 (SPSS Inc., Chicago, IL). Categorical variables were compared using Pearson's chi-squared tests. Logistic regression models were used to calculate odds ratios (OR) with 95% confidence intervals (CI) to compare those with single infection to those with co-detection. Dependent variables were symptom (e.g. presence of cough or rhinorrhoea) and outcome variables (e.g. hospitalisation or use of antibiotics).

We calculated adjusted ORs (aORs) by including the following covariates in the logistic regression models: age, gender, Aboriginal status, prematurity, presence of comorbidities, out-of-home care and household smoking. Age was included as a categorical variable in the models and were divided into 6-11 months, 12-23 months, 2 years, 3 years and 4 years (reference group). Covariates were selected based on known epidemiological or clinical risk factors for co-detection. Data from all patients were included in the adjusted models unless they had missing data on one or more covariates. To investigate the impact of specific pathogen pairs, analyses were repeated for the most common pathogen pairs. Estimated marginal means of logistic regression models were used to calculate probabilities with 95% CIs for antibiotic prescribing and hospitalisation for common pathogen pairs.

2.4 Ethical approvals

This study was approved by the PMH Human Research Ethics Committee (1673/EP), the Western Australian Aboriginal Health Ethics Committee (212 06/08) and the University of Western Australia Research Ethics Committee (RA/4/1/6456).

3 Results

Of 2715 patients recruited from 2008 to 2012, data for 2356 patients were available for analysis. Reasons for exclusion included incorrect or unknown age (n=154, 42.9% of all excluded patients), recruitment from general practice in 2008-2009 (n=131, 36.5%) , incomplete pathogen testing (n=29, 8.1%), unknown vaccination history (n=7, 1.9%), incomplete data (n=12, 3.3%), multiple enrolments for the same episode of illness (n=3, 0.8%), withdrawal from the study (n=23, 6.4%).

Of the 2356 patients enrolled, the majority (n=1848, 78.4%) were enrolled while presenting to PMH emergency department. Of these 6.3% (n=117) were subsequently admitted to hospital. The median age was 22.0 months (interquartile range=14.0-35.0), 54.9% were male and 5.7% were of Aboriginal or Torres Strait Islander decent. Children born preterm accounted for 13.5% (n=319) of patients. Children with comorbidities accounted for 15.1% (n=355) of this cohort. Of those that had one or more comorbidity, asthma (n=218, 61.4%) and other chronic respiratory conditions (n=54, 15.2%) were most common.

Of 2356 patients, 52.8% (n=1244) completed questions relating to outcomes (e.g. antibiotics use). Although parents were requested to complete these questions 7-10 days post-enrolment, yet the mean time to completion was 19.3 days and ranged

from 0 to 149 days (median=10 days). Data on antibiotic prescription post-enrolment were available for 51.0% (n=1201) of patients, of whom 483 (40.2%) were prescribed antibiotics. Combining data from questionnaires and review of hospital records resulted in near-complete data on hospitalisation (99.4%, n=2341), of whom 610 (26.1%) were hospitalized. Of those who were admitted to hospital, the median length of stay was 2 days (interquartile range=1-3).

Overall, 1630 patients (69.2%) tested positive for a virus. Of those with at least one virus detected, the most common were rhinovirus (n=665, 40.8%), influenza (n=481, 29.5%) and RSV (n=431, 26.4%; Figure 1). Of those with a virus detected, 24.8% (n=404) had at least one other virus co-detected. Of these, 350 (86.6%) had 2 viruses detected, 52 (12.9%) had 3 viruses detected and the remainder with 4 or more viruses.

A greater proportion of children with multiple viruses detected were less than 2 years old (65.4%) compared to those with a single virus infection (51.2%, p<0.001; Table 1). Those with co-detection also had greater odds of presenting with cough and rhinorrhoea compared to those with single infection, although both symptoms were common in both groups (Table 2). This effect remained after adjusting for other covariates. Of note, although less common, diarrhoea was more frequently observed in children with viral co-detection. There were no significant differences in the odds of being prescribed antibiotics (aOR=1.1, 95%CI: 0.8,1.5) or hospitalised (aOR=1.1, 95%CI: 0.8,1.4) between patients with single infection and co-detection (Table 2).

We then selected the three most common pathogens (rhinovirus, influenza and RSV) and investigated associations of specific pathogen pairs with antibiotic prescription and hospitalisation. After adjusting for other covariates, patients with both influenza and RSV detected had a 52% probability (95% CI:28%-76%) of being prescribed antibiotics with a trend towards more frequent prescription when compared with those with influenza or RSV infection alone (Figure 2). Similarly, the probability of being hospitalised was highest in those with influenza and RSV detected (probability=55%, 95% CI:35-73%); significantly greater when compared with those with influenza infection alone (probability=22%, 95%CI:16-29%; Figure 3) and with a trend observed compared with RSV infection alone (probability=43%, 95%CI:36-51%).

4 Discussion

This is one of the largest, single-site prospective studies of children up to 4 years of age that specifically investigates the incidence of and clinical outcomes associated with viral-viral co-detection. Our findings demonstrate that although differences in demographics, risk factors and symptoms are identifiable, in general, viral-viral co-detection is unlikely to be associated with more severe clinical illness among young children with influenza-like illness. Specific pathogen pairs may be associated with an increased probability of hospitalisation as was observed with influenza and RSV. This finding has implications in paediatric healthcare facilities where isolation of all children with acute respiratory viral infection is difficult during periods of peak respiratory virus activity and cohorting of children is frequently required prior to the availability of diagnostic test results.

We detected small differences in the symptoms presented by patients with single infection compared to those with co-detection. However, these symptoms were common and therefore, likely to be of little clinical relevance. On the other hand, the clinical outcomes chosen (i.e. antibiotics use and hospitalisation) were more indicative of disease severity but may be subject to clinical judgement and therefore, be less sensitive measures of disease severity. Accordingly, we observed no significant differences in the outcomes for children with single infection and those with co-detection.

This is consistent with data from previous systematic reviews, which found negligible differences between outcomes in children and adults with co-detection compared to peers with single infection [18,19]. However, further analyses by pathogen pairs suggest that some combinations of specific viral pathogens, such as influenza and RSV, are potentially more significant than others. This corroborates data from our recently completed systematic review that specifically investigated clinical outcomes in children with co-detection and found no differences overall but suggest that some pathogen specific effects may be present [20]. Our data suggest that future research in this area should segregate analysis by specific pathogen pairs where numbers allow.

We chose to exclude bocavirus and enterovirus detections from the analyses as their pathogenicity in ARTI is still not well-established. Bocavirus is often implicated in both symptomatic and asymptomatic co-detection and is thought to have a prolonged period of shedding [6]; both features which may confound any associations between co-detection and clinical severity. On the other hand, studies

on the role of enteroviruses in ARTI are suggestive of pathogenicity [21], however the numbers are small. For these reasons, detections of both viruses were excluded from the analyses presented here. Repeat analyses including these viruses did not change the overall findings (see Supplementary Tables 1 and 2).

An important consideration when interpreting these findings is the inability to distinguish between active (and pathogenic) infection and viral shedding. Prolonged viral shedding for some respiratory viruses, particularly rhinovirus, have been well-documented [22,23]. Quantitative analyses may be of assistance in distinguishing these clinical states yet has not become commonplace in the diagnostic laboratory for respiratory viruses.

One limitation of our study is that only children presenting to one hospital with influenza-like illness and fever were eligible for enrolment in this study. Consequently, it is possible that these children were at the more severe end of the disease spectrum which may bias our results. During the course of this study, there was a shift from using an antigen-based assay to using PCR when detecting hMPV, although both methods were used throughout the study period. We have elected to include detections from both methods but acknowledge that differences in the performance of these methods would mean that potential cases of hMPV may have been missed in earlier samples. These changes, as well as clinical discretion in testing for hMPV, may explain the lower proportions of hMPV detections in this cohort compared to other studies [24,25].

Further limitations of this study include missing outcomes data, particularly for antibiotic prescription. In addition, data on diagnosis at discharge were not collected, which may have helped to indicate the severity of symptoms. Moreover, despite enrolling nearly 2500 children, the number of patients with infections with specific pathogens and pathogen-pairs were relatively small.

Future studies using routinely collected, linked administrative data may assist in addressing both issues. Nonetheless, this is one of the largest single-site studies specifically investigating the effects of co-detection in young children using a wide panel of respiratory pathogens. Our results are similar to those reported elsewhere, adding to the validity of the findings [26].

We conclude that the impact of co-detection on disease severity in children presenting with influenza-like illness is likely to be limited to specific pathogen pairs. Therefore, routine screening for co-detection in this population should be restricted to common respiratory pathogens and efforts to reduce cross infection should focus on these specific pathogens.

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6 Conflicts of interest

None to declare.

7 Acknowledgements

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9 Tables

9.1 Table 1 – Cohort characteristics by infection status

Description	Frequency (n=2356)								
	No pathogen (n=726)			Single infection (n=1226)			Co-detection (n=404)		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
Aged less than 2 years	382	52.62	(48.91-56.30)	628	51.22	(48.38-54.06)	264	65.35	(60.48-69.98)
Male	397	54.68	(50.98-58.35)	668	54.49	(51.65-57.30)	228	56.44	(51.44-61.33)
Aboriginal or Torres Strait Islander descent	33	4.55	(3.15-6.32)	71	5.79	(4.55-7.25)	31	7.67	(5.27-10.71)
Born preterm	102	14.05	(11.60-16.79)	158	12.89	(11.06-14.89)	59	14.60	(11.31-18.43)
One or more comorbidities	117	16.12	(13.51-19.00)	183	14.93	(12.98-17.05)	55	13.61	(10.42-17.35)
More than 4 hours in out-of-home care	442	60.88	(57.22-64.45)	825	67.29	(64.59-69.91)	299	74.01	(69.44-78.22)
Smoking in household	154	21.21	(18.29-24.37)	283	23.08	(20.75-25.55)	107	26.49	(22.24-31.07)
Influenza vaccine on year of admission	188	25.90	(22.74-29.24)	303	24.71	(22.32-27.23)	100	24.75	(20.62-29.26)

Note: CI=Confidence Intervals. Exact 95% CI presented. Denominators include cases with missing data. Detections of enterovirus or bocavirus were ignored in counts of single and co-detection.

9.2 Table 2 – Frequency and logistic regression models of symptoms and outcomes by infection type

Description	Frequency		Logistic regression models	
	Single infection (n=1226)	Co-detection (n=404)	Co-detection	Co-detection
	% (95% CI)	% (95% CI)	OR [a] (95% CI)	aOR [a,b] (95% CI)
Symptoms				
Cough	88.66 (86.75-90.38)	93.32 (90.43-95.55)	1.95 (1.24-3.06)	1.94 (1.21-3.13)
Rhinorrhoea	88.09 (86.15-89.85)	93.32 (90.43-95.55)	2.07 (1.32-3.23)	1.79 (1.12-2.85)
Wheezing	43.56 (40.76-46.39)	49.01 (44.03-54.00)	1.26 (1.01-1.58)	1.20 (0.94-1.52)
Dyspnoea	45.84 (43.02-48.68)	50.74 (45.75-55.72)	1.23 (0.98-1.55)	1.15 (0.91-1.47)
Rash	17.86 (15.76-20.12)	14.11 (10.86-17.89)	0.75 (0.55-1.03)	0.69 (0.49-0.95)
Diarrhoea	20.39 (18.17-22.76)	27.23 (22.94-31.85)	1.47 (1.13-1.90)	1.33 (1.01-1.74)
Vomiting	38.58 (35.85-41.37)	42.82 (37.94-47.81)	1.19 (0.94-1.50)	1.16 (0.91-1.48)
Outcomes				
Antibiotics given [c]	19.98 (17.78-22.33)	21.53 (17.62-25.87)	1.19 (0.86-1.63)	1.11 (0.79-1.54)
Admitted to hospital	24.55 (22.16-27.06)	26.24 (22.01-30.82)	1.13 (0.87-1.46)	1.09 (0.83-1.44)

Note: CI=confidence intervals. Denominators include those with missing data. The number of children with missing data are as follows: cough (n=4 for single infection; n=3 for co-detection), rhinorrhoea (n=4; n=3), wheezing (n=4; n=4), dyspnoea (n=5; n=4), rash (n=30; n=9), diarrhoea (n=30; n=10), vomiting (n=32; n=9), antibiotics given (n=587; n=199), admitted to hospital (n=6; n=2). Infections with either enterovirus or bocavirus were ignored in counts of single infection and co-detection.

[a] Models presented are the odds of having a symptom/outcome in children with co-detection compared with children with single infection.

[b] Models were adjusted for age, gender, Aboriginal status, preterm birth, presence of comorbidities, out-of-home care and household smoking. All covariates listed were inputted as categorical variables.

[c] Data were only available for 639 children with single infection and 205 children with co-detection.

10 Figure legends

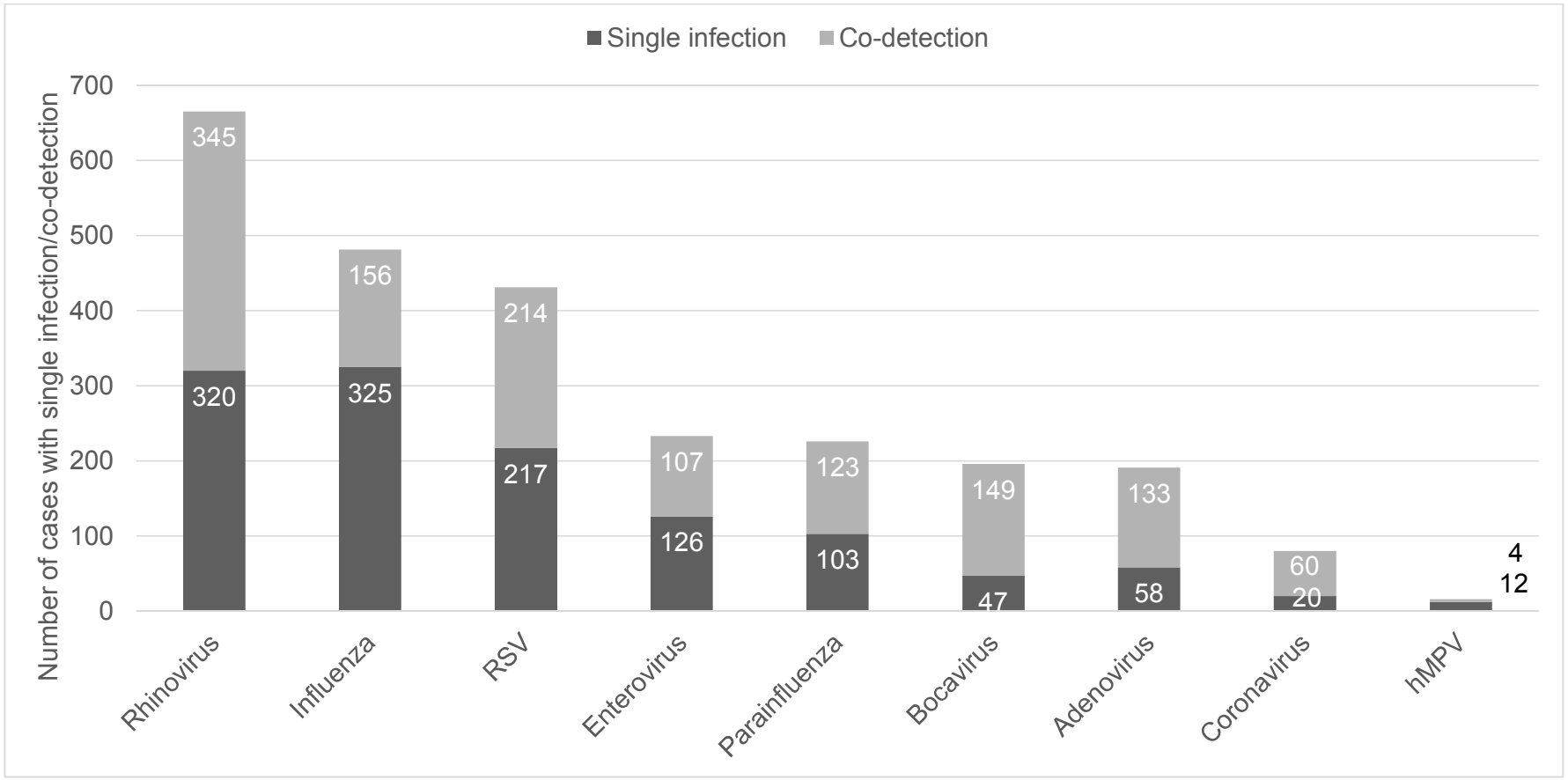
Figure 1 – Frequency of pathogen detection and co-detection

Figure 2 – Probability of post-enrolment antibiotics use by pathogen pairs with 95% confidence intervals

Figure 3 - Probability of hospitalisation by pathogen pairs with 95% confidence intervals

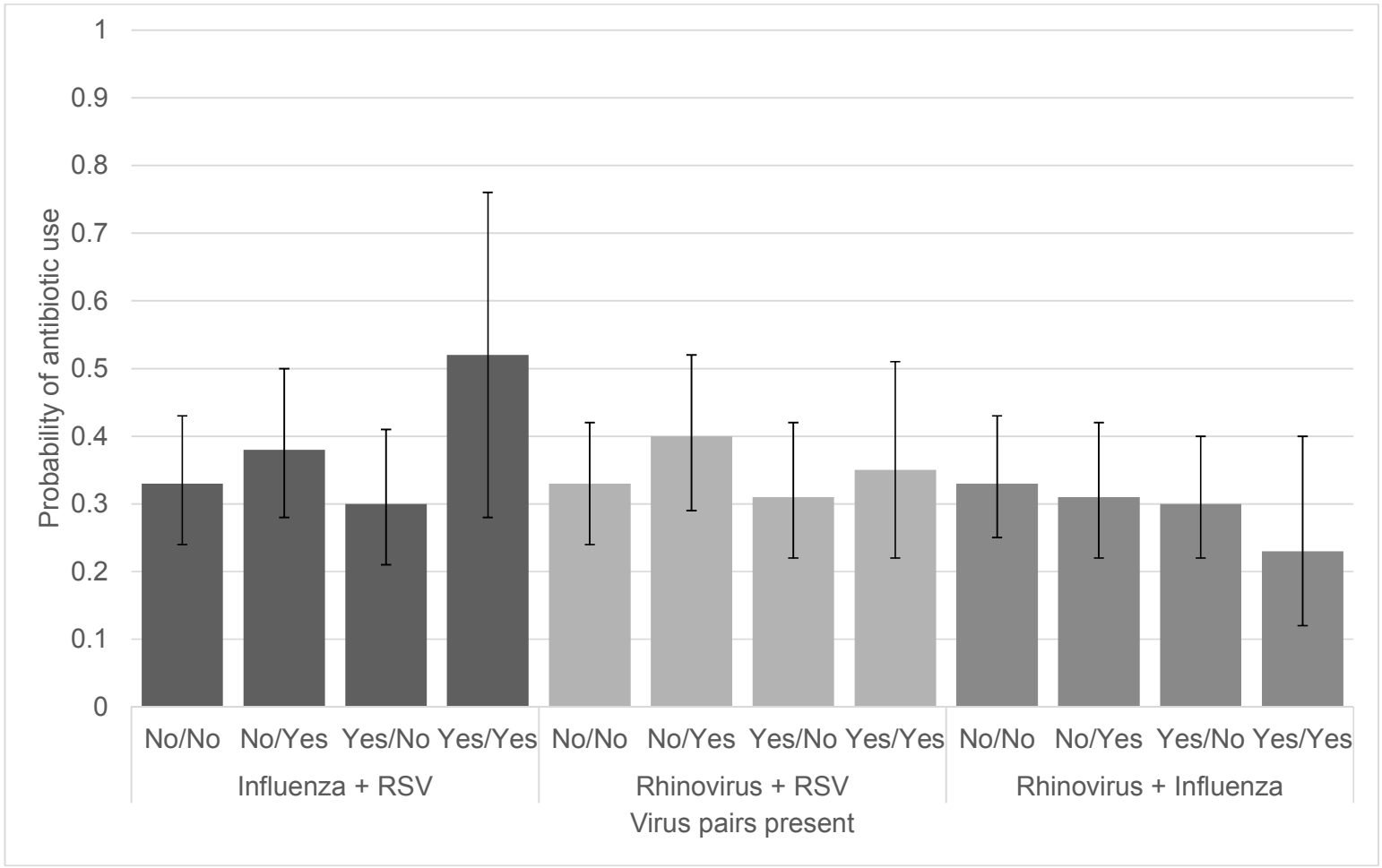
11 Figures

11.1 Figure 1 – Frequency of pathogen detection and co-detection



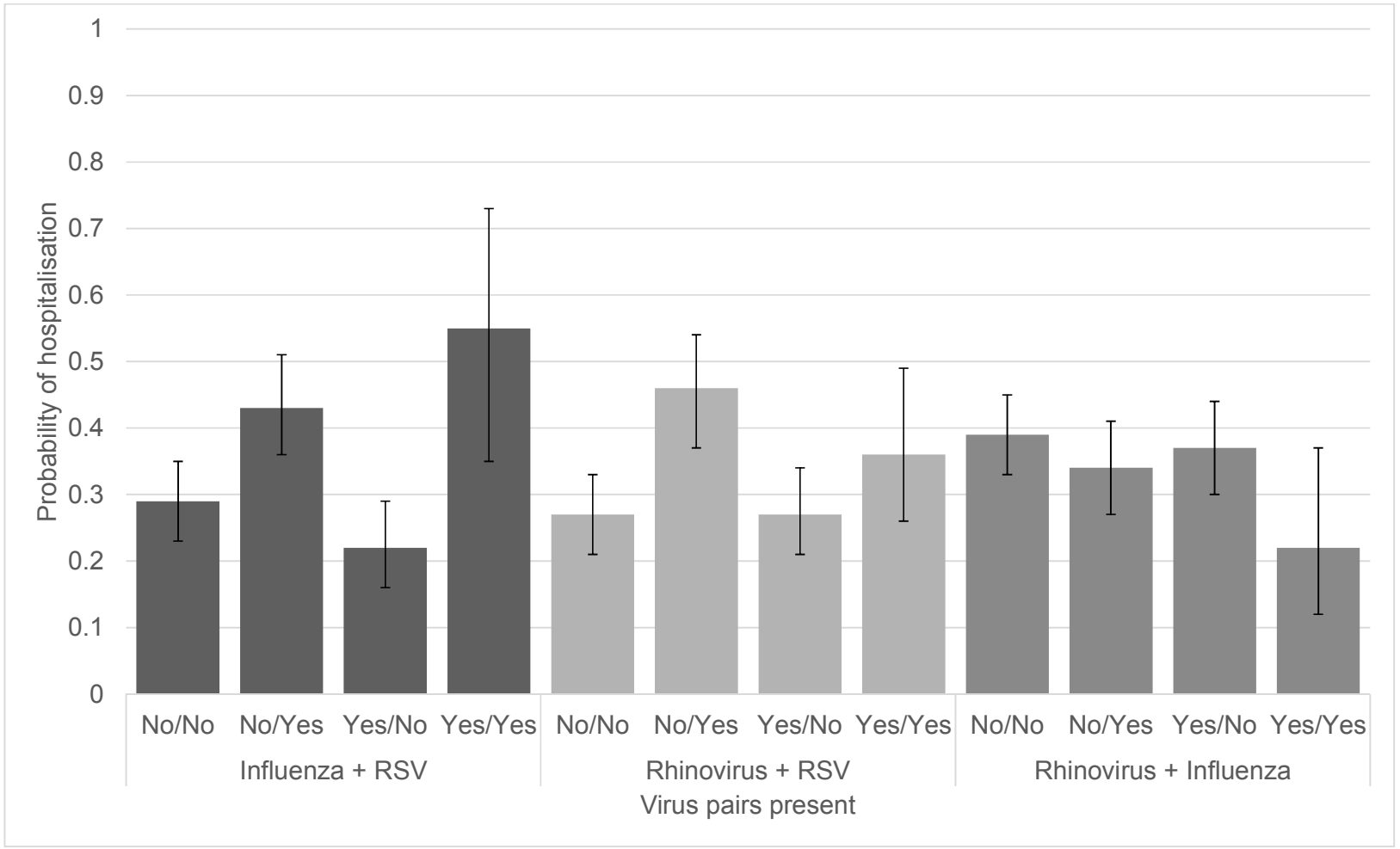
Note: RSV=respiratory syncytial virus, hMPV = human metapneumovirus. Detections of enterovirus and bocavirus were excluded from subsequent analyses.

11.2 Figure 2 – Probability of post-enrolment antibiotics use by pathogen pairs with 95% confidence intervals



Note: RSV=respiratory syncytial virus.

11.3 Figure 3 – Probability of hospitalisation by pathogen pairs with 95% confidence intervals



Note: RSV=respiratory syncytial virus.

Supplementary Table 1 – Cohort characteristics by infection status (all viruses included)

Description	Frequency (n=2356)								
	No pathogen (n=542)			Single infection (n=1228)			Co-detection (n=586)		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
Aged less than 2 years	279	51.48	(47.18-55.76)	611	49.76	(46.91-52.59)	384	65.53	(61.52-69.38)
Male	293	54.06	(49.76-58.31)	664	54.07	(51.24-56.89)	336	57.34	(53.22-61.38)
Aboriginal or Torres Strait Islander descent	26	4.80	(3.16-6.95)	72	5.86	(4.62-7.33)	37	6.31	(4.48-8.60)
Born preterm	70	12.92	(10.21-16.03)	163	13.27	(11.42-15.30)	86	14.68	(11.91-17.80)
Has 1 or more comorbidities	92	16.97	(13.91-20.40)	186	15.15	(13.19-17.28)	77	13.14	(10.51-16.15)
More than 4 hours in out-of-home care	346	63.84	(59.63-67.89)	812	66.12	(63.40-68.77)	408	69.62	(65.72-73.33)
Has smoking in household	119	21.96	(18.54-25.68)	282	22.96	(20.64-25.42)	143	24.40	(20.98-28.09)
Had influenza vaccine on year of admission	150	27.68	(23.95-31.65)	306	24.92	(22.52-27.44)	135	23.04	(19.69-26.66)

Note: CI=Confidence Intervals. Exact 95% CI presented. Denominators include cases with missing data.

Supplementary Table 2 – Frequency and logistic regression models of symptoms and outcomes by infection type (all viruses)

Description	Frequency		Logistic regression models	
	Single infection (n=1228)	Co-detection (n=586)	Co-detection	Co-detection
	% (95% CI)	% (95% CI)	OR [a] (95% CI)	aOR [a,b] (95% CI)
Symptoms				
Cough	87.87 (85.91-89.64)	91.47 (88.91-93.60)	1.53 (1.09-2.16)	1.63 (1.12-2.36)
Rhinorrhoea	86.64 (84.61-88.50)	92.32 (89.86-94.34)	1.94 (1.36-2.76)	1.72 (1.18-2.51)
Wheezing	43.08 (40.29-45.90)	48.63 (44.52-52.77)	1.26 (1.03-1.54)	1.23 (0.99-1.53)
Dyspnoea	44.87 (42.06-47.70)	51.19 (47.06-55.31)	1.30 (1.07-1.58)	1.27 (1.03-1.57)
Rash	17.59 (15.50-19.84)	15.70 (12.85-18.90)	0.87 (0.66-1.13)	0.82 (0.62-1.08)
Diarrhoea	19.95 (17.75-22.30)	24.57 (21.14-28.27)	1.31 (1.03-1.65)	1.17 (0.91-1.50)
Vomiting	38.93 (36.19-41.72)	40.44 (36.44-44.54)	1.06 (0.86-1.29)	1.03 (0.84-1.28)
Outcomes				
Antibiotics given [c]	19.71 (17.52-22.04)	20.99 (17.76-24.51)	1.09 (0.82-1.44)	0.98 (0.73-1.31)
Admitted to hospital	24.35 (21.97-26.85)	28.16 (24.55-31.99)	1.22 (0.97-1.52)	1.18 (0.92-1.49)

Note: CI=confidence intervals. Denominators include those with missing data. The number of children with missing data are as follows: cough (n=4 for single infection; n=3 for co-detection), rhinorrhoea (n=4; n=3), wheezing (n=4; n=4), dyspnoea (n=5; n=4), rash (n=32; n=13), diarrhoea (n=33; n=14), vomiting (n=34; n=13), antibiotics given (n=601; n=283), admitted to hospital (n=6; n=2).

[a] Models presented are the odds of having a symptom/outcome in children with co-detection compared with children with single infection.

[b] Models were adjusted for age, gender, Aboriginal status, preterm birth, presence of comorbidities, out-of-home care and household smoking. All covariates listed were inputted as categorical variables.

[c] Data were only available for 627 children with single infection and 283 children with co-detection.

Title

Viral etiology and the impact of ~~coinfection~~co-detection in young children presenting with influenza-like illness

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Keywords

Respiratory infection, Viral infection, ~~Coinfection~~Co-detection, Child

Running title

Viral etiology and impact of ~~coinfection~~co-detection

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Key points

- ~~Coinfection with respiratory virus occurs frequently in c~~Children frequently had multiple respiratory viruses detected
- Although common, children with multiple viruses more frequently had cough and rhinorrhoea ~~were more frequent in coinfected children~~
- ~~Those~~ Children with influenza and ~~+~~ respiratory syncytial virus were most frequently ~~tended to be~~ hospitalised
- Routine screening and cohorting recommended only for common respiratory pathogens

Author contributions

CCB, PCR, PVE and DWS conducted the WAIVE study. PCR, DWS and CCB conceptualised thi~~s~~e study. AL and ST conducted the laboratory work. ZVW and NTC conducted the preliminary data cleaning and analyses. FJL conducted data cleaning and analyses with assistance from HCM, NdK and CCB. FJL and ZVW jointly wrote the first draft of the manuscript. All authors have critically revised and approved of the final version of this manuscript.

Word counts

Abstract – ~~249~~ 248 words

Manuscript – Approx. 3~~2~~000 words

Abstract

Background

~~Viral coinfection in c~~Children with acute respiratory tract infection (ARTI) ~~is~~ frequently ~~report~~exhibit viral-viral co-detection~~ed~~, yet its clinical significance remains contentious. Using data from a prospective cohort of children with influenza-like illness, we described the virology of ARTI and determined the clinical impact of viral-viral ~~coinfection~~co-detection.

Methods

Children aged 6-59 months presenting to a tertiary paediatric hospital with fever and acute respiratory symptoms were enrolled and nasal samples collected ~~in an influenza vaccine effectiveness study~~ during influenza seasons in ~~from~~ 2008 ~~to~~ 2012. Respiratory v~~V~~iruses were identified by culture and PCR ~~from nasal swabs~~. We compared ~~the~~ demographics, presenting symptoms and clinical outcomes of children with single viral infection and viral-viral ~~coinfection~~co-detection. We ~~then~~ used logistic regression models and estimated marginal means to calculate the adjusted odds ratio and probabilities of symptom presentation, antibiotic prescription or hospitalisation~~, adjusted for demographic factors~~.

Results

~~Of 2487 eligible patients, 1728~~ 1630 of 2356 children (69.2%) had a virus detected, among whom rhinovirus (40.8%), influenza (29.5%) and respiratory syncytial virus (RSV; 26.4%) were most commonly detected. 24% of these had ~~of which 24% were coinfected with~~ two or more viruses detected. After ~~. Rhinovirus (40%), influenza (29%) and respiratory syncytial virus (RSV; 27%) were the most commonly detected~~

viruses. aAdjusting for other demographic factors, children with co-detection coinfection had greater odds of presenting with cough (aOR=2.01.9, 95% CI:=1.32-3.1.2), and rhinorrhoea (aOR=1.98, 95% CI:=1.21-2.9) than those with single infection, although both symptoms were common. Children with Comparing virus-virus combinations, influenza and RSV combined was associated withhad the the highest st probability of antibiotics prescription (probability=58%, 95%CI=33-79%) and hospitalisation (probability=554%, 95%CI:=354-73%), significantly greater than those with influenza infection alone (probability=22%, 95%CI:16-29%).

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Conclusions

Overall, coinfectionco-detection has limited impact on clinical severity among children with influenza-like illness. However, specific pathogen pairs may be associated with more severe outcomes. Routine diagnostics to identify viral Future research should segregate by pathogen where feasible. Routine screening for coinfectionco-detection should be restricted to common pathogens.

1 Introduction

Acute respiratory tract infections (ARTI) in children place a significant burden on families and the community. Commonly recognized respiratory viral pathogens include influenza viruses, respiratory syncytial viruses (RSV), parainfluenza viruses, human rhinoviruses, adenoviruses and coronaviruses [1,2]. Advances in laboratory diagnostic techniques have resulted in the discovery of new viruses, including human metapneumovirus (hMPV) and polyomaviruses [3,4], yet a number of these pathogens have ~~. These advances have also enabled greater delineation between pathogenic viruses and viruses of~~ uncertain pathogenicity [5,6].

~~Coinfection~~Co-detection can be defined as detection of two or more pathogens in a single sample. With the improved sensitivity, availability and affordability of modern diagnostics, viral-viral ~~coinfection~~co-detections are being increasingly identified. The incidence of viral-viral ~~coinfection~~co-detection has been reported between 15-45%, depending on age, location and testing methods [7–9]. The clinical significance of ~~coinfection~~co-detection in ARTI remains contentious with the literature ranging ~~as current evidence ranges~~ from negligible to deleterious effects [9,10].

This study describes the virology of ARTI in children aged six months to four years who~~,~~ presented~~ing to a paediatric emergency department~~ with influenza-like illness during influenza season to a tertiary paediatric hospital in Australia. This study also enabled us to specifically examine~~, and determines~~ the impact of viral-viral ~~coinfection~~co-detection on clinical symptoms and outcomes.

2 Materials and Methods

2.1 Study setting and patients

Western Australia (WA) spans 2.5 million square kilometres with a population of approximately 2.5 million people, 7% of whom are under 5 years of age [11]. Princess Margaret Hospital for Children (PMH) is the only tertiary paediatric hospital in the state and is located in metropolitan Perth where approximately 80% of the population resides [12].

Commencing in 2008, t~~T~~he Western Australia Influenza Vaccine Effectiveness (WAIVE) Study was an observational cohort study established to determine the effectiveness of inactivated influenza vaccine~~commenced in 2008~~. Patient recruitment was conducted at PMH (and at selected general practises in metropolitan WA in 2008-2009). Due to small numbers recruited and differences in presentation, data from children presenting to general practises were removed from these analyses.

Patient recruitment coincided with the annual influenza seasons. ~~and is an observational study conducted during the annual influenza season.~~ The start and end of influenza seasons were defined by the Infectious Diseases Surveillance Unit at PathWest Laboratory Medicine WA using a combination of indicators, including weekly proportion of laboratory influenza tests positive. As a guide, two consecutive weeks with over 10% influenza test positive often coincides with the beginning of influenza season in WA. Further details on ~~The~~ study design are ~~is~~ described elsewhere [13].

~~In summary, A~~all children 6-59 months of age presenting to PMH with a history of fever (by parental report) or with a measured temperature of greater than 37.5°C at presentation, accompanied by at least one acute respiratory symptoms within the previous 96 hours, were eligible for enrolment. All children transited through PMH emergency department. A portion of these children were subsequently admitted to hospital with the remainder discharged home from the emergency department. ~~While the bulk of recruitment occurred at PMH emergency department, children may also be recruited from PMH inpatient facilities or from general practices (2008 only). Influenza seasons each year were determined by influenza surveillance data in the state [13].~~ Children with a known immunodeficiency disorder, current or recent immunosuppressive treatment, or who received immunoglobulin in the previous three months were excluded from the study.

Patient demographics, medical history and presenting symptoms were collected by parental questionnaire. Comorbidities recorded included prematurity, asthma, chronic cardiac, neurological or respiratory conditions. Influenza vaccination status was obtained by parental report and confirmed through the Australian Childhood Immunisation Register or by contacting immunisation providers. Vaccination status for other vaccines were not collected. A follow-up questionnaire of illness outcomes, including details of hospital admission(s), use of antibiotics and time to recovery, was provided to families to complete within 7-10 days after enrolment. A retrospective review of medical records was undertaken when hospitalisation data were incorrectly recorded or missing. No follow-up was conducted for antibiotics use if data were missing.

2.2 Respiratory virus detection

Children had ~~a~~ bilateral mid-turbinate ~~nasopharyngeal~~ nasal swabs ~~or aspirate~~ collected at enrolment (Copan Diagnostics Inc., Murrieta, CA). If a nasopharyngeal aspirate had already been collected by hospital staff as part of clinical care, this sample was used in lieu of a nasal swab. Viral culture (Madin-Darby Canine Kidney cells, Diploid lung fibroblasts) and multiplex tandem PCR was used to detect all viruses except ~~enterovirus, rhinovirus~~picornaviruses and hMPV [14,15]. ~~Enteroviruses and rhinoviruses~~Picornaviruses were detected using nested PCR [16] targeting the 5'UTR of the picornavirus genome with sequencing used to assist with identification of rhinoviruses and enteroviruses~~speciation~~. hMPV was tested using an immunofluorescent assay (Simulfluor hMPV Immunofluoresent Assay; Millipore, Temecula, CA) and PCR. All patients were subjected to the same panel of tests and testing methods were consistent throughout the study period with the exception of testing for hMPV; testing for hMPV was based on clinical need. While both immunofluorescence and PCR assays were used throughout the study period, PCR testing was more common in later years.

For all viruses (except hMPV), positive viral detection was defined as detection by viral culture and/or PCR. Positive detection of hMPV was defined as detection by immunofluorescence and/or PCR. All influenza types/subtypes (i.e. influenza A/H1N1, A/H3N2 and B) were grouped for analysis. Similarly, s~~S~~ubgroups of ~~influenza viruses and~~ parainfluenza viruses (i.e. parainfluenza types 1-4) were grouped together for analysis. Infection was defined as detection of one or more of rhinovirus, influenza, RSV, parainfluenza, adenovirus, coronavirus or hMPV.

~~Coinfection~~Co-detection was defined as detection of two or more viruses in a single diagnostic sample.

2.3 Definitions and statistical analysis

Prematurity was defined as less than 37 weeks of gestation at birth. Out-of-home care was defined as attendance at playgroup, mothers' group, day-care centre, kindergarten or preschool ~~for four or more hours per week~~. Length of stay in hospital refers to the duration from admission to discharge date. Symptoms investigated included cough, rhinorrhoea, wheeze, dyspnoea, rash, diarrhoea and vomiting while outcomes investigated were antibiotic prescription and hospital admission.

Data cleaning and analyses were performed in Microsoft Excel, EpiBasic [17] and SPSS version 23~~2~~ (SPSS Inc., Chicago, IL). Categorical variables were compared using Pearson's chi-squared tests. Logistic regression models were used to calculate odds ratios (OR) with 95% confidence intervals (CI) to compare those with single infection to those with ~~coinfection~~co-detection. Dependent variables were symptom (e.g. presence of cough or rhinorrhoea) and outcome variables (e.g. hospitalisation or use of antibiotics).

We calculated adjusted ORs (aORs) by including the following covariates in the logistic regression models: age, gender, Aboriginal status, prematurity, presence of comorbidities, out-of-home care and household smoking. Age was included as a categorical variable in the models and were divided into 6-11 months, 12-23 months, 2 years, 3 years and 4 years (reference group). Covariates were selected based on known epidemiological or clinical risk factors for co-detection. ~~Records with missing~~

~~data for any of the covariates were excluded from the fully adjusted models.~~ Data from all patients were included in the adjusted models unless they had missing data on one or more covariates. To investigate the impact of specific pathogen pairs, analyses were repeated for the most common pathogen pairs. Estimated marginal means ~~Post-hoc analysis~~ of ~~selected~~ logistic regression models were~~as~~ used to calculate probabilities with 95% CIs for antibiotic prescribing and hospitalisation for common pathogen pairs.

2.4 Ethical approvals

This study was approved by the PMH Human Research Ethics Committee (1673/EP), the Western Australian Aboriginal Health Ethics Committee (212 06/08) and the University of Western Australia Research Ethics Committee (RA/4/1/6456).

3 Results

Of 2715 patients recruited from 2008 to 2012, data for ~~2487~~ 2356 patients were available for analysis. Reasons for exclusion included incorrect or unknown age (n=154, ~~67.5~~42.9% of all excluded patients), recruitment from general practice in 2008-2009 (n=131, 36.5%) , incomplete pathogen testing ~~not completed~~ (n=29, ~~12.7~~8.1%), unknown vaccination history (n=7, ~~3.1~~1.9%), incomplete data (n=12, ~~5.3~~3.3%), multiple enrolments for the same episode of illness (n=3, ~~1.3~~0.8%), ~~or~~ withdrawal from the study (n=23, ~~10.1~~6.4%).

Of the 2356 patients enrolled, the majority (n=1848, 78.4%) were enrolled while presenting to PMH emergency department. Of these 6.3% (n=117) were subsequently admitted to hospital. ~~Of those included in analyses, T~~the median age

was 22.0 months (interquartile range=14.0-356.0), 554.9.2% were male and 5.67% were of Aboriginal or Torres Strait Islander decent. Children born preterm accounted for 13.35% (n=319) of patients. Children with comorbidities accounted for 15.1% (n=355) of this cohort. Of those that had one or more comorbidity, asthma (n=21825, 62.061.4%) and other chronic respiratory conditions (n=54, 15.2%, 14.9%) were most common.

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Of 2356 patients, 52.8% (n=1244) completed questions relating to outcomes (e.g. antibiotics use). Although parents were requested to complete these questions 7-10 days post-enrolment, yet the mean time to completion was 19.3 days and ranged from 0 to 149 days (median=10 days). Data on antibiotic prescription post-enrolment were available for 51.052.6% (n=13081201) of patients, of whom 542483 (41.440.2%) were prescribed antibiotics. Combining data from questionnaires and review of hospital records resulted in near-complete data on hospitalisation (99.4%, n=2341), Hospitalisation data were available for 99.0% (n=2462) of patients, of whom 596 610 (26.14.2%) were hospitalized. Of those who were admitted to hospital, the median length of stay was 2 days (interquartile range=1-3).

Overall, 1630728 patients (69.569.2%) tested positive for a virus. Of those with at least one virus detected, the most , most common werely rhinovirus (n=665, 40.8%), influenza (n=481, 29.5%) and RSV (n=431, 26.4%; Figure 1). Of those with at least onea virus detected, 24.84% (n=404) had at least one other virus co-detectedwere coinfected. Of these, 36750 (86.687.1%) had were infected with 2 viruses detected,

52 (12.94%) had ~~were infected with~~ 3 viruses detected and the remainder with 4 or more viruses.

A greater proportion of ~~C~~children with multiple viruses ~~coinfection~~detected were less than 2 years old (65.4%) ~~younger (median age=18.0 months, interquartile range=12.0-29.0) and attended out-of-home-care more~~ compared to those with a single virus infection (51.2%, p<0.001~~median age=23.0 months, interquartile range=14.0-37.5~~; Table 1). Those with ~~coinfection~~co-detection also had greater ~~double the~~ odds of presenting with cough and rhinorrhoea compared to those with single infection, although both symptoms were common in both groups (Table 2). This effect remained after adjusting for other covariates. Of note, although less common, diarrhoea was more frequently observed in children with viral co-detection. There were no significant differences in the odds of being prescribed antibiotics (aOR=1.1, 95%CI: 0.8,1.5) or hospitalised (aOR=1.1, 95%CI: 0.8,1.4) between patients with single infection and ~~coinfection~~co-detection (Table 2).

We then selected the three most common pathogens (rhinovirus, influenza and RSV) and investigated associations of specific pathogen pairs with antibiotic prescription and hospitalisation. After adjusting for other covariates, patients ~~coinfected~~ with both ~~an~~ influenza and ~~/~~RSV ~~pair~~ detected had a 528% probability (95% CI:~~=~~2833%-796%) of being prescribed antibiotics with a trend towards more frequent prescription when compared with those with influenza or RSV infection alone (Figure 2). Similarly, the probability of being hospitalised was highest in those ~~coinfected~~ with influenza and RSV detected (probability=554%, 95% CI:~~=~~345%-73%); significantly greater when compared with those with influenza infection alone (probability=2~~7~~2%,

95%CI:=156%-289%;) with a non-significant trend observed when compared with those with RSV infection alone (probability=41%, 95%CI=34%-49%). No other differences were noted when comparing coinfection and single infection with other pathogen pairs (Figure 3) and with a trend observed compared with RSV infection alone (probability=43%, 95%CI:36-51%).

4 Discussion

This is one of the largest, single-site prospective studies of children up to 4 years of age that specifically investigates the incidence of and clinical outcomes associated with viral-viral coinfectionco-detection. Our findings demonstrate that although significant differences in demographics, risk factors and symptoms are identifiable, in general, viral-viral coinfectionco-detection is unlikely to be associated with more severe clinical illness among young children with influenza-like illness. However S, specific pathogen pairs may be associated with an increased probability of hospitalisation as was observed with influenza and RSV. This finding has significant implications in paediatric healthcare facilities where isolation of all children with acute respiratory viral infection is difficult during periods of peak respiratory virus activity and cohorting of children is frequently required prior to the availability of diagnostic test results.

We detected small differences in the symptoms presented by patients with single infection compared to those with coinfectionco-detection. However, these symptoms were common and therefore, likely to be of little clinical relevance. On the other hand, the clinical outcomes chosen (i.e. antibiotics use and hospitalisation) were more indicative of disease severity but may beare subject to clinical judgement and

therefore, ~~may~~ be less ~~in~~sensitive measures of disease severity. Accordingly, we observed no significant differences in the outcomes for children with single infection and those with ~~coinfection~~co-detection.

This is consistent with data from previous systematic reviews, which found negligible differences between outcomes in children and adults with ~~coinfection~~co-detection compared to peers with single infection [18,19]. However, further analyses by pathogen pairs suggest that some ~~virus~~ combinations of specific viral pathogens, such as influenza and RSV, are potentially more ~~pathogenic~~ significant than others. This corroborates data from our recently completed systematic review that specifically investigated clinical outcomes in children with ~~coinfection~~co-detection and found no differences overall but suggest that some pathogen specific effects may be present ~~(PROSPERO registration: CRD#42014009133).~~[20]. Our data suggest that future research in this area should segregate analysis by specific pathogen pairs where numbers allow.

We chose to exclude bocavirus and enterovirus detections from the analyses as their pathogenicity in ARTI is still not well-established. Bocavirus is often implicated in both symptomatic and asymptomatic ~~coinfection~~co-detection and is thought to have a prolonged period of shedding [6]; both features which may confound any associations between ~~coinfection~~co-detection and clinical severity. On the other hand, studies on the role of enteroviruses in ARTI are suggestive of pathogenicity [21], however the numbers are small. For these reasons, detections of both viruses were excluded from the analyses presented here. Repeat analyses including these viruses did not change the overall findings (see Supplementary Tables 1 and 2).

An important consideration when interpreting these findings is the inability to distinguish between active (and pathogenic) infection and viral shedding. Prolonged viral shedding for some respiratory viruses, particularly rhinovirus, have been well-documented [22,23]. Quantitative analyses may be of assistance in distinguishing these clinical states yet has not become commonplace in the diagnostic laboratory for respiratory viruses.

~~We attempted to investigate the relationship between rhinovirus infection and clinical severity [22–24]. However, we were limited as we were unable to subtype rhinoviruses; in particular, rhinovirus-C has been associated with greater respiratory illness than subtype A [5]. Based on the proportion of patients who were hospitalised with influenza and rhinovirus infections in this cohort, data from approximately 7700 children would have been required to provide 80% power of detecting a difference, at the 0.05 level of significance, between those with a rhinovirus-C infection and those with an influenza virus infection.~~

One limitation of our study is that only children presenting to one hospital with influenza-like illness and fever were eligible for enrolment in this study. Consequently, it is possible that these children were at the more severe end of the disease spectrum which may bias our results. During the course of this study, there was a shift from using an antigen-based assay to using PCR when detecting hMPV, although both methods were used throughout the study period. We have elected to include detections from both methods but acknowledge that differences in the performance of these methods would mean that potential cases of hMPV may have

been missed in earlier samples. These changes, as well as clinical discretion in testing for hMPV, may explain the lower proportions of hMPV detections in this cohort compared to other studies [24,25].

Further limitations of this study include missing outcomes data, particularly for antibiotic prescription. ~~.~~ In addition, data on diagnosis at discharge were not collected, which may have helped to indicate the severity of symptoms. Moreover, despite enrolling nearly 2500 children, the number of patients with infections with specific pathogens and pathogen-pairs were relatively small.

Future studies using routinely collected, linked administrative data may assist in addressing both issues. Nonetheless, this is one of the largest single-site studies specifically investigating the effects of ~~coinfection~~co-detection in young children using a wide panel of respiratory pathogens. Our results are similar to those reported elsewhere, adding to the validity of the findings [26].

We conclude that the impact of ~~coinfection~~co-detection on disease severity in children presenting with influenza-like illness is likely to be limited to specific pathogen pairs. Therefore, routine screening for ~~coinfection~~co-detection in this population should be restricted to common respiratory pathogens and efforts to reduce cross infection should focus on these specific pathogens.

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6 Conflicts of interest

None to declare.

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9 Tables

9.1 Table 1 – Cohort characteristics by infection status

Description	Frequency (n=~~2487~~2356)								
	No pathogen (n=~~726~~759)			Single infection (n=~~1226~~1307)			~~Coinfection~~Co-detection (n=~~404~~421)		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
Aged less than 2 years	~~382~~392	52.62 ~~51.65~~	(48.91-~~48.03-55.26~~)56.30)	~~628~~6 ~~72~~	51.22~~5~~ 1.42	(48.38-54.06)~~(48.67-54.16)~~	~~264~~ 271	65.35~~6~~ 4.37	(60.48-69.98)~~(59.59-68.95)~~
Male	~~397~~414	54.68 ~~54.55~~	(50.98-58.35~~(50.93-58.13)~~)	~~668~~7 ~~17~~	54.49~~5~~ 4.86	(51.65-57.30)~~(52.11-57.58)~~	~~228~~ ~~241~~	56.44~~5~~ 7.24	(51.44-61.33)~~(52.36-62.02)~~
Aboriginal or Torres Strait Islander descent ~~descent~~	~~33~~34	4.55~~4.48~~	~~(3.12-6.20)~~(3.15-6.32)	~~71~~74	5.79~~5.66~~	(4.55-7.25)~~(4.47-7.06)~~	~~313~~ ~~1~~	7.67~~7.36~~	(5.27-10.71)~~(5.06-10.29)~~
Born preterm	~~102~~106	14.05	(11.60-	~~158~~1	12.89~~1~~	(11.06-	~~596~~	14.60~~1~~	(11.31-

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		13.97	16.79(11.58-16.64))	65	2.62	14.89)(10.87-14.55)	0	4.25	18.43)(11.06-17.96)
One or more comorbidities	117418	16.12	(13.51-	1831	14.931	(12.98-	555	13.611	(10.42-
		15.55	19.00(13.04-18.32))	89	4.46	17.05)(12.60-16.49)	6	3.30	17.35)(10.21-16.92)
More than 4 hours in out-of-home care	442459	60.88	(57.22-	8258	67.296	(64.59-	299	74.017	(69.44-
		60.47	64.45(56.90-63.97))	72	6.72	69.91)(64.09-69.27)	307	2.92	78.22)(68.41-77.11)
Smoking in household	154165	21.21	(18.29-	2833	23.082	(20.75-	107	26.492	(22.24-
		21.74	24.37(18.85-24.85))	05	3.34	25.55)(21.07-25.73)	110	6.13	31.07)(21.99-30.60)
Influenza vaccine on year of admission	188213	25.90	(22.74-	3033	24.712	(22.32-	100	24.752	(20.62-
		28.06	29.24(24.89-31.41))	64	7.85	27.23)(25.43-30.37)	111	6.37	29.26)(22.22-30.85)

Note: CI=Confidence Intervals. Exact 95% CI presented. Denominators include cases with missing data. Detections of enterovirus or bocavirus were ignored in counts of single and coinfectionco-detection.

9.2 Table 2 – Frequency and logistic regression models of symptoms and outcomes by infection type

Description	Frequency				Logistic regression models			
	Single infection (n=1226)		Co-detection (n=404)		Co-detection		Co-detection	
	%	(95% CI)	%	(95% CI)	OR [a]	(95% CI)	aOR [a,b]	(95% CI)
Symptoms								
Cough	88.66	(86.75-90.38)	93.32	(90.43-95.55)	1.95	(1.24-3.06)	1.94	(1.21-3.13)
Rhinorrhoea	88.09	(86.15-89.85)	93.32	(90.43-95.55)	2.07	(1.32-3.23)	1.79	(1.12-2.85)
Wheezing	43.56	(40.76-46.39)	49.01	(44.03-54.00)	1.26	(1.01-1.58)	1.20	(0.94-1.52)
Dyspnoea	45.84	(43.02-48.68)	50.74	(45.75-55.72)	1.23	(0.98-1.55)	1.15	(0.91-1.47)
Rash	17.86	(15.76-20.12)	14.11	(10.86-17.89)	0.75	(0.55-1.03)	0.69	(0.49-0.95)
Diarrhoea	20.39	(18.17-22.76)	27.23	(22.94-31.85)	1.47	(1.13-1.90)	1.33	(1.01-1.74)
Vomiting	38.58	(35.85-41.37)	42.82	(37.94-47.81)	1.19	(0.94-1.50)	1.16	(0.91-1.48)
~~At least 1 symptom~~	~~97.55~~	~~(96.53-98.34)~~	~~99.26~~	~~(97.85-99.85)~~				
Outcomes								
Antibiotics given [c,b]	19.98	(17.78-22.33)	21.53	(17.62-25.87)	1.19	(0.86-1.63)	1.11	(0.79-1.54)

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Admitted to hospital	~~23.98~~24.55 (~~21.61-26.47~~22.16-27.06)	26.24	(22.01-30.82)	1.13	(0.87-1.46)	1.09	(0.83-1.44)

Note: CI=confidence intervals. Denominators include those with missing data. The number of children with missing data are as follows: cough (n=4 for single infection; n=3 for co-detection), rhinorrhoea (n=4; n=3), wheezing (n=4; n=4), dyspnoea (n=5; n=4), rash (n=30; n=9), diarrhoea (n=30; n=10), vomiting (n=32; n=9), antibiotics given (n=587; n=199), admitted to hospital (n=6; n=2).

Infections with either enterovirus or bocavirus were ignored in counts of single infection and ~~coinfection~~co-detection.

~~Adjusted models were adjusted for age, gender, Aboriginal status, preterm birth, presence of comorbidities, out-of-home care and household smoking. Models presented are the odds of having a symptom/outcome in children with coinfection compared with children with single infection.~~

[a] Models presented are the odds of having a symptom/outcome in children with co-detection compared with children with single infection.

[b] Models were adjusted for age, gender, Aboriginal status, preterm birth, presence of comorbidities, out-of-home care and household smoking. All covariates listed were inputted as categorical variables.

~~[a]~~[c] Data were only available for ~~706~~ 639 children with single infection and ~~219~~ 205 children with ~~coinfection~~co-detection.

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10 Figure legends

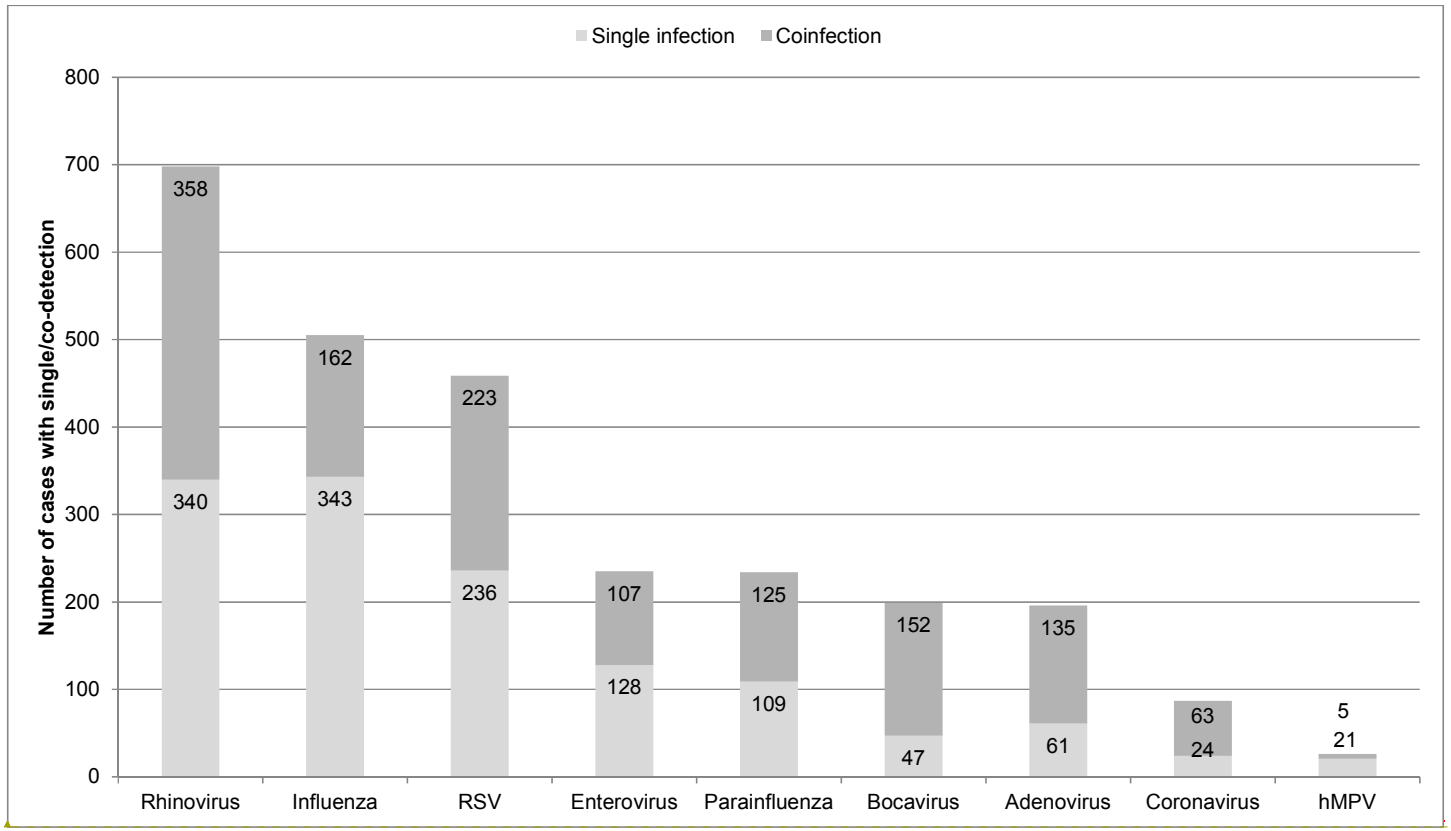
Figure 1 – Frequency of pathogen detection and ~~coinfection~~co-detection

Figure 2 – Probability of post-enrolment ~~use of~~ antibiotics use by pathogen pairs with 95% confidence intervals

Figure 3 - Probability of hospitalisation by pathogen pairs with 95% confidence intervals

11 Figures

11.1 Figure 1 – Frequency of pathogen detection and ~~coinfection~~co-detection



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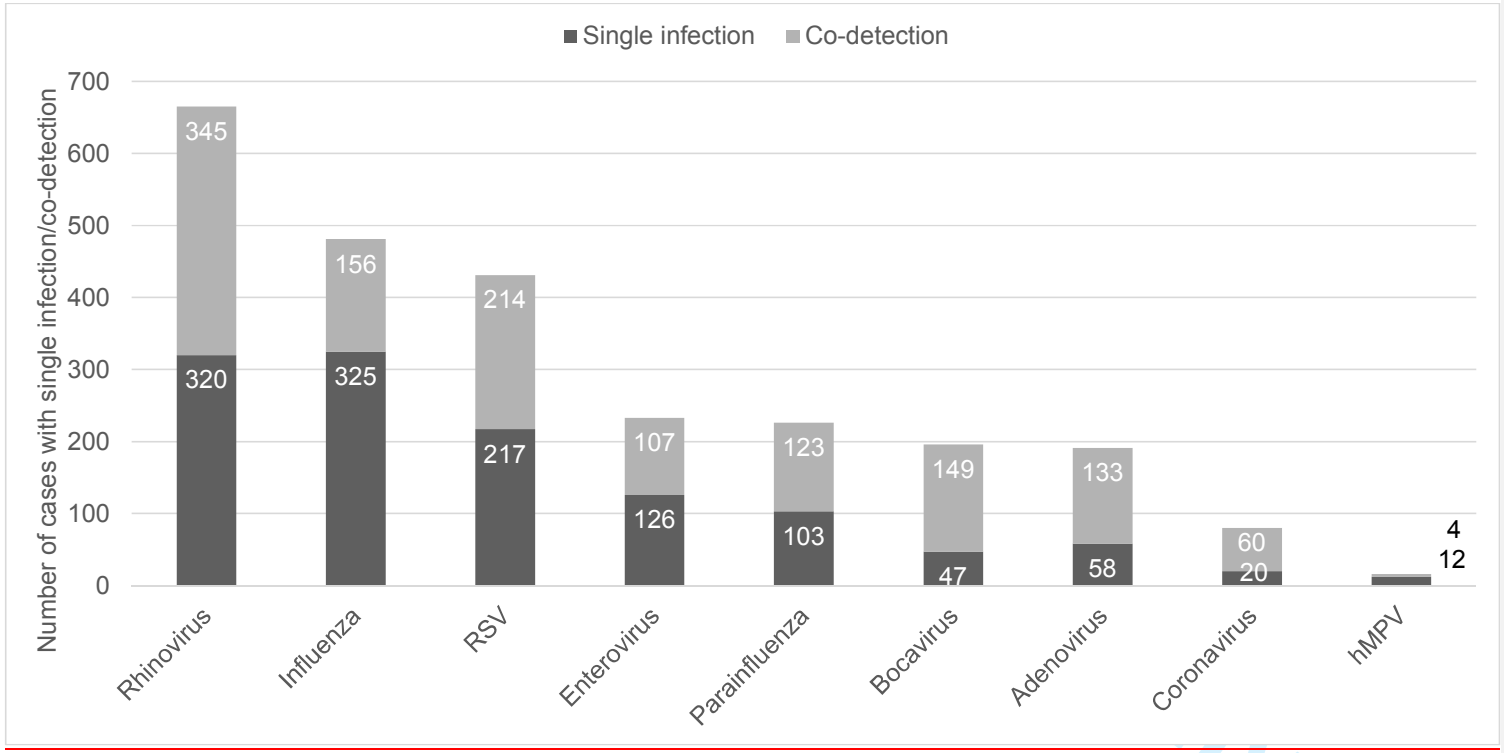
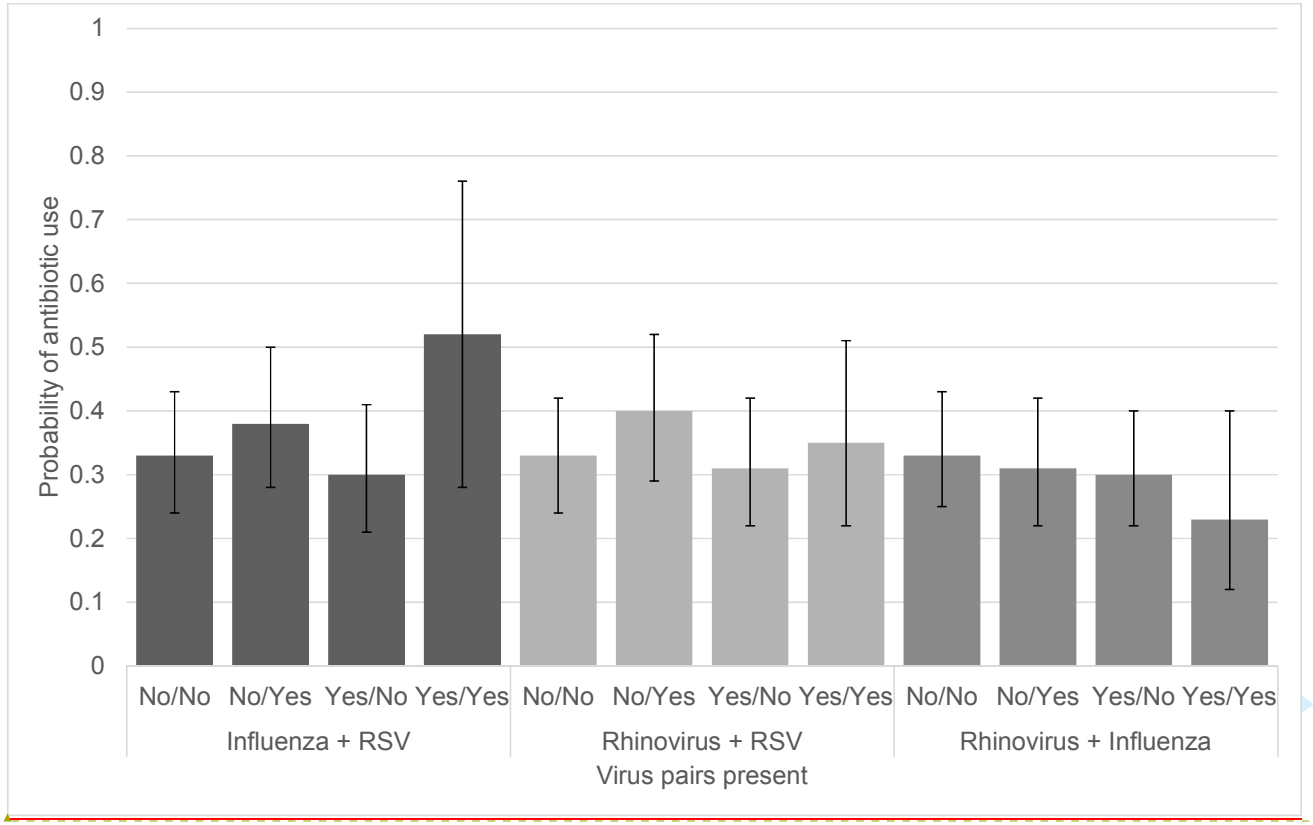


Note: RSV=respiratory syncytial virus, hMPV = human metapneumovirus. Detections of enterovirus and bocavirus were excluded from subsequent analyses.

11.2 Figure 2 – Probability ~~of use~~ of post-enrolment antibiotics use ~~by~~ by pathogen pairs with 95% confidence intervals



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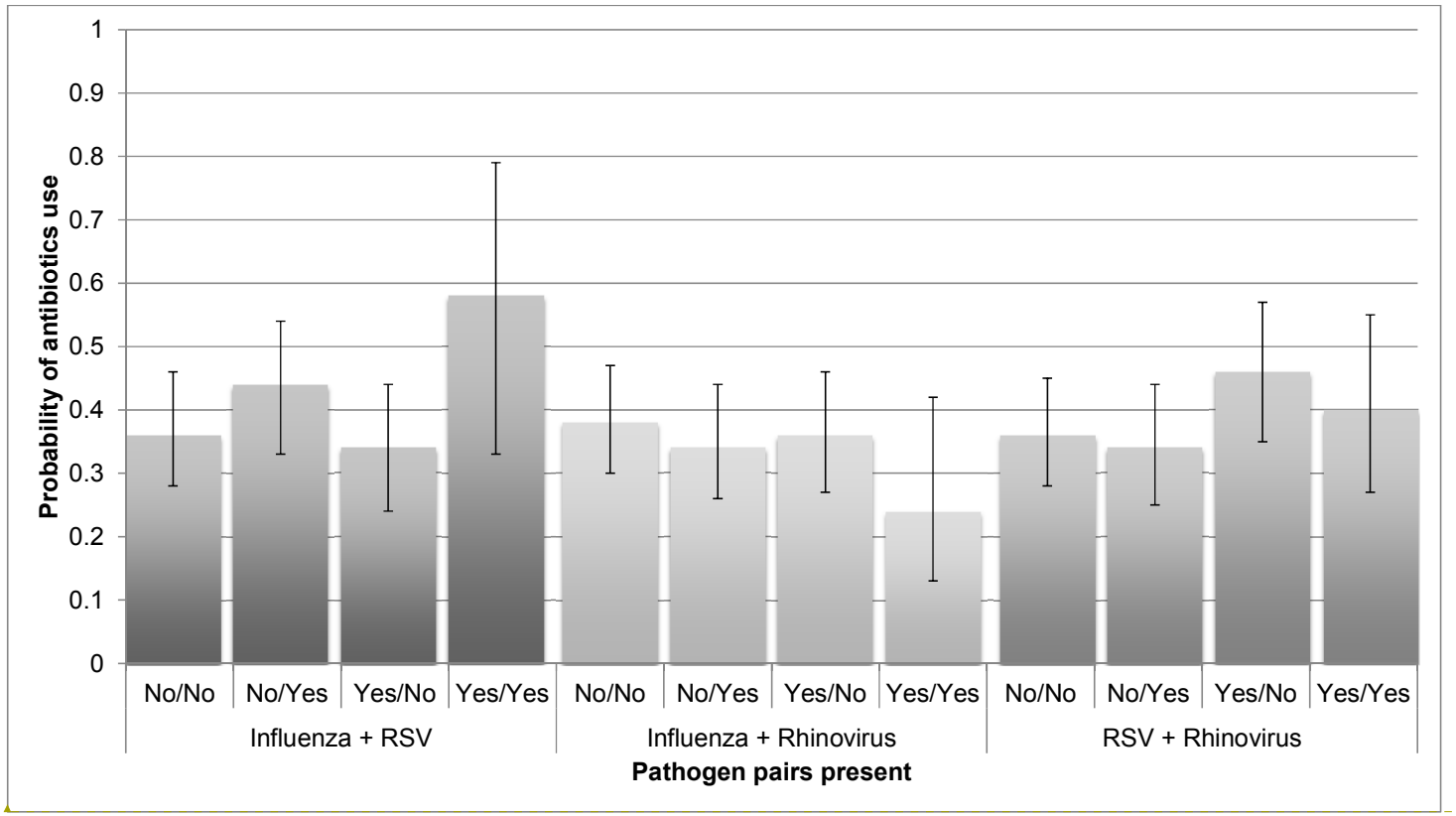

Note: RSV=respiratory syncytial virus.

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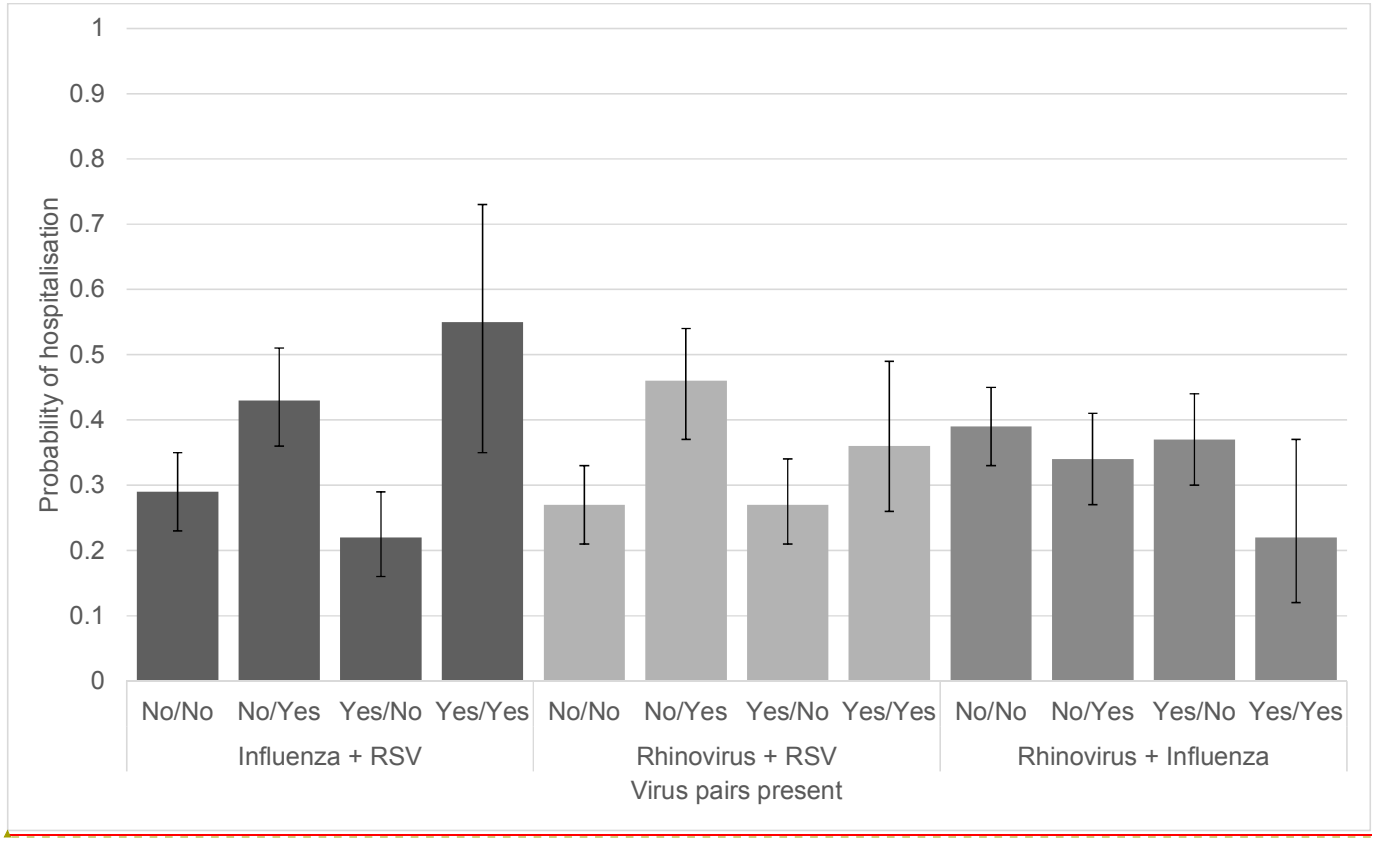
11.3 Figure 3 – Probability of hospitalisation by pathogen pairs with 95% confidence intervals



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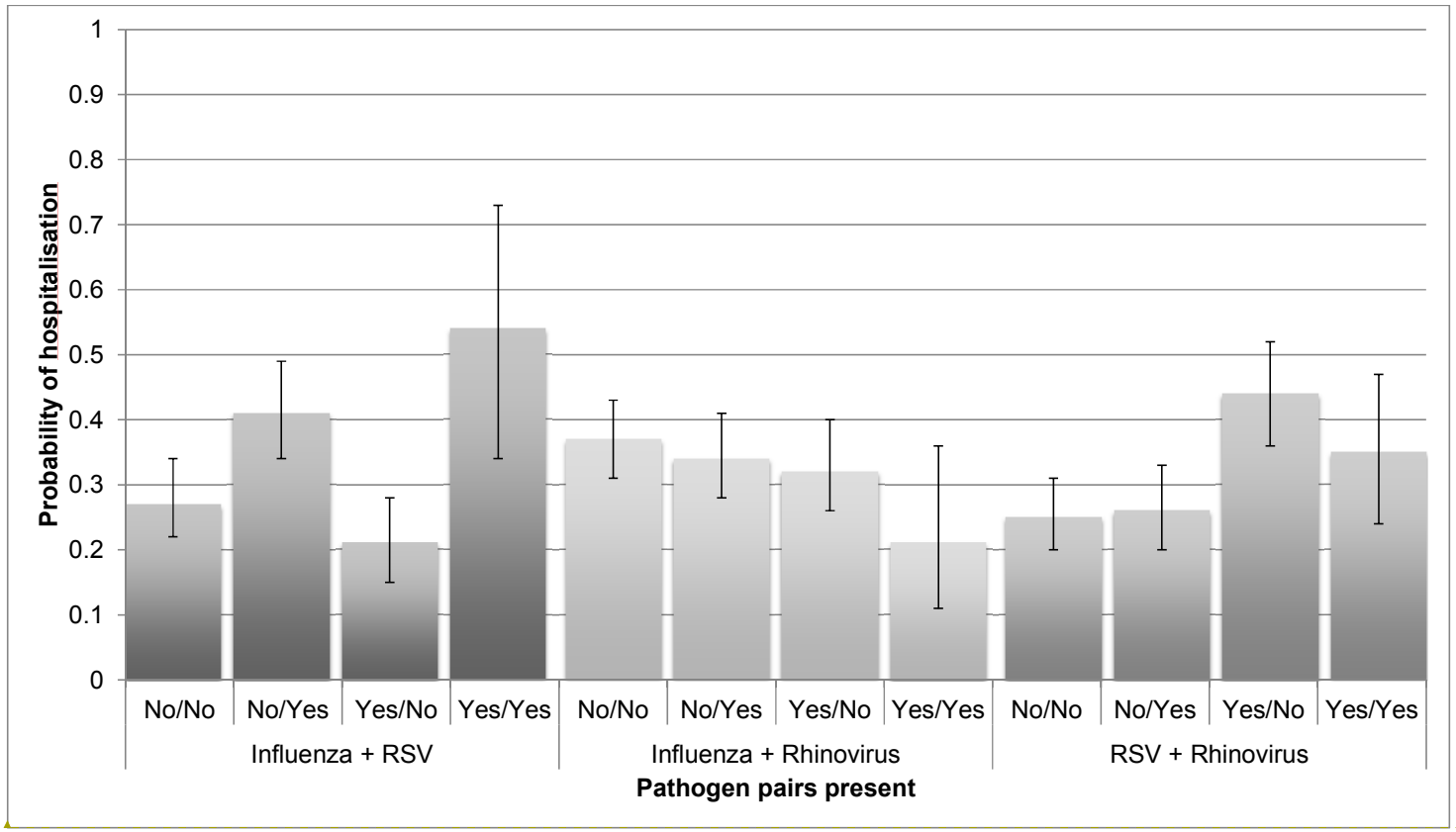

Note: RSV=respiratory syncytial virus.

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Supplementary Table 1 – Cohort characteristics by infection status (all viruses included)

Description	Frequency (n=23562487)								
	No pathogen (n=542573)			Single infection (n=12281309)			Co-detectioninfection (n=586605)		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
Aged less than 2 years	279288	51.485 0.26	(47.18-55.76)(46.09-54.43)	6116 54	49.764 9.96	(46.91-52.59)(47.22-52.71)	384 393	65.53 64.96	(61.52-69.38)(61.01-68.76)
Male	293310	54.065 4.10	(49.76-58.31)(49.92-58.24)	6647 12	54.075 4.39	(51.24-56.89)(51.65-57.12)	336 350	57.34 57.85	(53.22-61.38)(53.80-61.82)
Aboriginal or Torres Strait Islander descent	2627	4.804. 71	(3.16-6.95)(3.13-6.78)	7275	5.865. 73	(4.62-7.33)(4.53-7.13)	373 7	6.316 .12	(4.48-8.60)(4.34-8.33)
Born preterm	7074	12.921 2.91	(10.21-16.03)(10.28-	1631 69	13.271 2.91	(11.42-15.30)(11.14-	868 8	14.68 14.55	(11.91-17.80)(11.88-

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			15.94)			14.85)			17.61)
Has 1 or more comorbidities	9293	16.971 6.23	(13.91-20.40)(13.30-19.51)	1861 92	15.151 4.67	(13.19-17.28)(12.79-16.70)	777 8	13.14 12.89	(10.51-16.15)(10.33-15.83)
More than 4 hours in out-of-home care	346361	63.846 3.00	(59.63-67.89)(58.90-66.97)	8128 61	66.126 5.78	(63.40-68.77)(63.13-68.35)	408 416	69.62 68.76	(65.72-73.33)(64.90-72.43)
Has smoking in household	119130	21.962 2.69	(18.54-25.68)(19.32-26.34)	2823 04	22.962 3.22	(20.64-25.42(20.96-25.61))	143 146	24.40 24.13	(20.98-28.09)(20.77-27.75)
Had influenza vaccine on year of admission	150174	27.683 0.37	(23.95-31.65)(26.62-34.31)	3063 66	24.922 7.96	(22.52-27.44(25.54-30.48))	135 148	23.04 24.46	(19.69-26.66(21.09-28.09))

Note: CI=Confidence Intervals. Exact 95% CI presented. Denominators include cases with missing data.

Supplementary Table 2 – Frequency and logistic regression models of symptoms and outcomes by infection type (all viruses)

Description	Frequency				Logistic regression models			
	Single infection (n=~~1309~~1228)		Co-~~detection~~infection (n=~~605~~586)		Co-~~detection~~	~~Co~~infection	Co-~~detection~~	~~Co~~infection
	%	(95% CI)	%	(95% CI)	OR [a]	(95% CI)	aOR [a,b]	(95% CI)
Symptoms								
Cough	87.8~~87.55~~	(85.91-89.64)~~(85.64-89.29)~~	91.4~~91.24~~	(88.91-93.60)~~(88.70-93.37)~~	1.53~~1.54~~	(1.09-2.16)~~(1.10-2.16)~~	1.63~~1.65~~	(1.12-2.36)~~(1.15-2.37)~~
Rhinorrhoea	86.64~~86.78~~	(84.61-88.50)~~(84.83-88.57)~~	92.32~~92.40~~	(89.86-94.34)~~(89.99-94.38)~~	1.94~~1.96~~	(1.36-2.76)~~(1.37-2.79)~~	1.72~~1.75~~	(1.18-2.51)~~(1.21-2.54)~~
Wheezing	43.08~~42.63~~	(40.29-45.90)~~(39.93-45.36)~~	48.63~~47.93~~	(44.52-52.77)~~(43.89-52.00)~~	1.26~~1.25~~	(1.03-1.54)~~(1.03-1.52)~~	1.23~~1.22~~	(0.99-1.53)~~(0.99-1.50)~~
Dyspnoea	44.87~~44.23~~	(42.06-47.70)~~(41.52-~~	51.19~~50.25~~	(47.06-	1.30~~1.28~~	(1.07-	1.27~~1.24~~	(1.03-

		46.97)		55.31)(46.19-54.31)		1.58)(1.05-1.55)		1.57)(1.01-1.53)
Rash	17.5917.34	(15.50-19.84)(15.33-19.50)	15.7015.37	(12.85-18.90)(12.59-18.50)	0.870.86	(0.66-1.13)(0.66-1.12)	0.820.81	(0.62-1.08)(0.62-1.07)
Diarrhoea	19.9520.63	(17.75-22.30)(18.46-22.96)	24.5724.46	(21.14-28.27)(21.07-28.09)	1.311.24	(1.03-1.65)(0.99-1.56)	1.171.11	(0.91-1.50)(0.87-1.41)
Vomiting	38.9338.35	(36.19-41.72)(35.71-41.05)	40.4440.66	(36.44-44.54)(36.72-44.70)	1.061.10	(0.86-1.29)(0.90-1.34)	1.031.07	(0.84-1.28)(0.87-1.32)
Outcomes								
Antibiotics given [c]	19.7121.62	(17.52-22.04)(19.42-23.95)	20.9921.65	(17.76-24.51)(18.43-25.15)	1.091.01	(0.82-1.44)(0.77-1.33)	0.980.92	(0.73-1.31)(0.70-1.22)
Admitted to hospital	24.3522.31	(21.97-26.85)(20.08-24.66)	28.1627.11	(24.55-31.99)(23.60-	1.221.29	(0.97-1.52)(1.03-	1.181.25	(0.92-1.49)(0.98-

	~~30.84)~~	~~1.61)~~	~~1.58)~~

Note: CI=confidence intervals. Denominators include those with missing data. The number of children with missing data are as follows: cough (n=4 for single infection; n=3 for co-detection), rhinorrhoea (n=4; n=3), wheezing (n=4; n=4), dyspnoea (n=5; n=4), rash (n=32; n=13), diarrhoea (n=33; n=14), vomiting (n=34; n=13), antibiotics given (n=601; n=283), admitted to hospital (n=6; n=2).

[a] Models presented are the odds of having a symptom/outcome in children with co-detection compared with children with single infection.

[b] Models were adjusted for age, gender, Aboriginal status, preterm birth, presence of comorbidities, out-of-home care and household smoking. All covariates listed were inputted as categorical variables.

[c] Data were only available for 627 children with single infection and 283 children with co-detection.

~~Adjusted models were adjusted for age, gender, Aboriginal status, preterm birth, presence of comorbidities, out-of-home care and household smoking. Models presented are the odds of having a symptom/outcome in children with coinfection compared with children with single infection.~~

~~[a] Data were only available for 694 cases with single infection and 319 cases with coinfection.~~