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Microbiological aetiology of paediatric respiratory tract infections in Kyrgyzstan

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Abstract

Introduction Respiratory tract infections (RTIs) are a leading cause of morbidity and mortality in children worldwide. Aetiologic virus and bacteria vary geographically. To date, no systematic data on RTI aetiologies have been published from Kyrgyzstan, a country with a high under-five mortality from lower RTIs and a widespread overuse of antibiotics. We aimed to identify the aetiologies of RTI in children in Kyrgyzstan and to assess the pneumococcal conjugate vaccine (PCV) vaccination rate and the correlation with the detection of *Streptococcus pneumoniae* in our population.

Methods We collected samples from children aged 6 months to 12 years presenting with symptoms of acute RTI for outpatients or diagnosed with pneumonia for inpatients. Samples were collected from November 2022 to June 2023 and were analysed using standard culture methods and real-time polymerase chain reaction (RT-PCR) to identify bacterial, viral, and atypical bacterial pathogens. We obtained the vaccination status through vaccination records and parents' recollections.

Results Positive bacteriologic cultures were found for 294 (25.0%) of 1174 outpatients and 83 (27.7%) of 300 inpatients. *S. pneumoniae* was the most commonly found bacterial pathogen in both groups, making up 44.8% of all cultured bacteria. Furthermore, 445 (64.1%) of 694 outpatients with RT-PCR performed had one or more viral pathogens identified, while this was the case for 162 (54.7%) of 296 inpatients. *Mycoplasma pneumoniae* was identified in 15.8% of inpatients possibly representing an epidemic in the spring of 2023. Approximately 20% of both in- and outpatients had a viral-bacterial simultaneous detection. 87.1% of outpatients and 75.9% of inpatients had been vaccinated with PCV. We found a higher proportion of unvaccinated children among those under five years of age and a higher carriage rate of *S. pneumoniae* among unvaccinated inpatients.

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Conclusion In this study, we found a high prevalence of viral infections and viral-bacterial simultaneous detections among Kyrgyz children with respiratory tract infections (RTI). The findings from this study can inform new clinical guidelines for local health workers and lead to more targeted use of antibiotics and improved health outcomes for children in Kyrgyzstan.

Clinical Trial Not applicable.

Key findings

- This study presents the first published data on the causes of respiratory tract infections in children in Central Asia.
- Viral pathogens were detected in 54.7% of inpatients and 64.1% of outpatients, with rhinovirus being the most common.
- Bacterial pathogens were identified by culture in 27.7% of inpatients and 25.0% of outpatients. The most frequent bacterium was *Streptococcus pneumoniae*.
- *Mycoplasma pneumoniae* was detected in 15.8% of inpatients, coinciding with an outbreak identified in March 2023.
- A significantly lower proportion of inpatients under five were vaccinated with the pneumococcal conjugate vaccine, with just 68.6% having received the vaccination doses according to age.

Background

Respiratory tract infections (RTIs) are the leading cause of healthcare visits among children globally and remain one of the primary causes of under-five mortality, particularly in low- and middle-income countries (LMIC) [1–3]. According to the Global Burden of Disease Study 2015, Kyrgyzstan reported one of the highest under-five mortality rates due to lower RTIs in Central and Eastern Europe and Central Asia, with an incidence of 133 per 100,000 per year [4]. Additionally, in Kyrgyzstan, the use of antibiotics is unregulated [5].

The most common pathogens causing RTIs worldwide are respiratory syncytial virus (RSV), human metapneumovirus (HMPV), influenza A and B, parainfluenza, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Mycoplasma pneumoniae* [3, 6–8]. Additionally, viral-bacterial co-infections contribute substantially to the burden of lower RTIs in children [8]. This picture varies geographically and over time and is changing with vaccination campaigns and the introduction of novel pathogens such as SARS-CoV-2 [2, 6, 9].

The pneumococcal conjugate vaccine (PCV) protects both vaccinated and unvaccinated children against invasive pneumococcal disease [10] and decreases nasopharyngeal carriage and transmission of *S. pneumoniae* [11]. In Kyrgyzstan, the 13-valent PCV (PCV13) is given at two, five, and 12 months. It was introduced in 2016, and in 2022 the immunization rate was estimated to be 92% [12]. Similarly, the *H. influenzae* type B (Hib) vaccine, implemented in Kyrgyzstan in 2009, reached a coverage rate of 90% in 2022 [12].

We aimed to gain systematic data on the aetiologies of respiratory tract infections in Kyrgyz children to support health recommendations and optimise antibiotic use. For this purpose, we identified bacterial and viral pathogens responsible for RTIs in paediatric inpatients and outpatients during the 2022–2023 RTI season in Kyrgyzstan. We also collected data on PCV13 vaccination coverage across our inpatient and outpatient populations and examined its correlation with the detection of *S. pneumoniae* in airway samples.

Materials and methods

This study included outpatients from two separate Regions, Naryn and Chui, and inpatients from two health facilities in the capital of Kyrgyzstan, Bishkek.

Data and sample collection - outpatients

The outpatient data collection was established as part of the randomised controlled trial 'C-reactive protein for Respiratory Diagnosis in Kyrgyz Paediatric Practice' (COORDINATE). COORDINATE tested the efficacy of point of care measurement of C-reactive protein as a safe additional diagnostic tool to reduce unnecessary antibiotic treatment in Kyrgyz children with RTI [13, 14].

Briefly, the data collection was conducted during normal working hours in 14 primary health clinics in the semi-urban region of Chui and the rural region of Naryn. Children aged 6 months to 12 years with symptoms of RTI were consecutively included upon consent from parents or caretakers. The inclusion criteria were less than 2 weeks with cough, fast/difficult breathing, sore throat, shortness of breath, or wheezing. Patients who reported having taken antibiotics within 24 h of the consultation were excluded. Patients were also excluded and referred to hospital if they were judged to be severely ill and in need of urgent hospitalisation. The outpatient study aimed for a sample size of 1204, adhering to that of the COORDINATE trial. Preliminary calculations established that this would allow for detection of

a pneumococcal positivity rate of 30% with a margin of error of 3%, an alfa of 5% and a dropout rate of 20%.

The children and caretakers were interviewed regarding the child's illness prior to clinical examination. The study assistants collected naso- or oropharyngeal swabs or both. The swabs were kept at 4–6 °C until transportation in a cooling box to the National Reference Laboratory under the Department of Disease Prevention and State Sanitary and Epidemiological Surveillance. The samples were transported daily from Chui and three times weekly from Naryn.

Outpatient data and sample collection were initiated in November 2022 and continued until the desired sample size was reached in March 2023.

Data and sample collection - inpatients

Inpatients were included during normal working hours from two clinics: The National Centre of Maternity and Childhood Care and the City Children's Clinical Hospital, both located in Bishkek. The inclusion criteria for inpatients were; age 6 months to 12 years and primary diagnosis of pneumonia. We did not exclude inpatients who had previously taken antibiotics.

We included children whose parents or caretakers provided oral and written consent. Medical history and clinical examination were carried out, and a sample was taken via a naso- or oropharyngeal swap or, when possible, by isolating sputum from a suction tube or by expectoration in older children. The samples were refrigerated on-site and transported daily to the National Reference Laboratory. Chest X-rays were assessed by the local radiologist. We aimed to include 300 inpatients. This was decided pragmatically based on resources, time, and knowledge of admission rates at the two centres.

Inpatient data and sample collection were initiated in December 2022 and continued until the desired sample size was reached in June 2023.

PCV13 vaccination

For both inpatients and outpatients, data on PCV13 were retrieved through vaccination books or through the recollection of the parent or guardian. If a child had had the appropriate PCV13 doses according to age [12], we considered the child to be covered.

Clinical examination

Patients were examined by trained health care workers. Oxygen saturation was measured with pulse oximetry. Temperature was measured with non-contact thermometers or in the armpit using mercury or digital thermometers.

Preparation for data collection

Before data collection, standard operating procedures for sampling, data recording, and laboratory procedures were developed, and study assistants and laboratory personnel were trained in these. All findings were recorded in case report forms developed for this study (available as supplementary material in English) in collaboration with the COORDINATE trial.

Bacteriological culture

All swabs were cultured at the National Reference Laboratory on 5% horse-blood agar, chocolate agar, 6.5% salt agar, and tellurite agar (all manufactured locally). Plates were incubated overnight (for a minimum of 20 h) and examined for growth of any of the following potential pathogens: Beta-haemolytic streptococci, *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, *S. aureus*, and *Corynebacterium diphtheriae.*

Additionally, we recorded growth in two or more streak zones of any other pathogen. Bacteria were identified by growth pattern, microscopy, and standard biochemical tests. We grouped beta-haemolytic streptococci according to Lancefield groups using latex agglutination (PathoDX™ Strep Grouping Kit, Thermo Fisher Scientific™, US).

Real-time polymerase chain reaction (RT-PCR)

We aimed to examine samples from 700 of the 1204 outpatients and from all 300 inpatients for virus and atypical bacterial agents by RT-PCR at the virology laboratory under the Department of Disease Prevention and State Sanitary and Epidemiological Surveillance, Bishkek. The selection of a subsample of 700 outpatients for RT-PCR was a pragmatic decision based on available resources. For logistical reasons, all swabs were frozen at -80 °C and subsequently thawed before analysis.

The samples were analysed using the DT-Prime 5 (DNA-Technology LLC, Moscow) with the following kits: AmpliPrime® Influenza SARS-CoV-2/Flu (detecting SARS-CoV-2, Influenza B, and Influenza A of the subtypes H1pm09, H3N2, H1N1, H5, H7, and H9), AmpliSens® Mycoplasma pneumoniae/Chlamydia pneumoniae, AmpliSens® Legionella pneumophila, and AmpliSens® ARVI-screen (detecting Human Respiratory Syncytial virus; Human Metapneumovirus; Human Parainfluenza virus 1–4; Human Rhinovirus 100 serotypes; Human Coronavirus E229, NL63, OC43, HKUI; Human Adenovirus B, C, E; and Human Bocavirus). All kits were from InterLabServices, (Moscow, Russia).

To assess the monthly variation in pathogen prevalence, the outpatient samples analysed via RT-PCR were distributed throughout the study period. Practically, we aimed to equalize the number of samples analysed per study week. When a week had more samples than

Table 1 Background data of 300 inpatients and 1174 outpatients with pneumoniae and respiratory tract infection

Background data	Inpatients, *N* = 300	Outpatients, *N* = 1,174	*p*-value[1]
Sex, male	156 (52.0%)	606 (51.6%)	0.906
< 1 y	55 (18.3%)	157 (13.4%)	*0.029*
1–3 y	147 (49.0%)	452 (38.5%)	*< 0.001*
4–6 y	50 (16.7%)	281 (23.9%)	*0.008*
7–9 y	23 (7.7%)	201 (17.1%)	*< 0.001*
10–12 y	25 (8.3%)	83 (7.1%)	0.454
Febrile (≥ 38 °C)[3]	43 (14.3%)	106 (9.0%)	*0.007*
Oxygen saturation < 93%[4]	76 (25.3%)	65 (5.5%)	*< 0.001*
Vaccination status	**Inpatients**, *N* = 278	**Outpatients**, *N* = 1126	**p-value[1]**
PCV13-vaccinated[2]	211 (75.9%)	981 (87.1%)	*< 0.001*
<5 y	140 (68.6%)	546 (80.3%)	
5–12 y	71 (95.9%)	435 (97.5%)	
p-value[1,2]	*< 0.001*	*< 0.001*	

From 300 inpatients diagnosed with pneumonia and 1174 outpatients with symptoms of respiratory tract infection in Kyrgyzstan. Patients were aged 6 months to 12 years

[1]Chi-square test

[2]For difference in PCV13 vaccination status between under-fives and 5-12-year-olds

[3]Temperature was measured with non-contact thermometers or in the armpit using mercury or digital thermometers

[4]Oxygen saturation was measured with pulse oximetry

Abbreviations: y, year; PCV13, 13-valent pneumococcal conjugate vaccine

Table 2 Selected presenting symptoms and findings of 300 paediatric inpatients diagnosed with pneumonia in Bishkek, Kyrgyzstan

Symptom/Finding	*N* = 300
Cough[1]	300 (100%)
Sore throat[1]	29 (9.7%)
Symptoms of rhinitis[1]	189 (63.0%)
Fast/difficult breathing[1]	228 (76.0%)
Wheezing[1]	62 (20.7%)
Indrawing of chest[2]	163 (53.3%)
Crepitation[2]	124 (21.3%)
Rhonchi[2]	138 (46.0%)
X-ray taken[3]	298 (99.3%)
Consolidation/infiltration	290 (97.3%)
Atelectasis	1 (0.3%)
Bilateral changes	56 (18.8%)
Pleural empyema	0 (0%)

The patients were admitted in two inpatient clinics in Bishkek, Kyrgyzstan, and aged 6 months to 12 years

[1]Reported in patient history

[2]As found on examination

[3]X-rays were described by local radiologists

desired, we selected those collected first during the week (e.g., Monday samples were prioritized over Tuesday samples).

Data management and analysis

Data were stored in paper form centrally behind locked doors at the Department of Respiratory Medicine and Intensive Care, Bishkek. We entered data into a central REDCap (Research Electronic Data Capture) database [15] hosted by the Capital Region of Denmark.

We analysed the data using R version 4.3.1. The bacteriological and virological findings were presented with descriptive statistics grouped by patient group and by age group (under-fives, 5–12-year-olds). Chi-square tests or Fisher's exact tests were used for group comparisons with a significance level of $p < 0.05$. For monthly variation in pathogen prevalence, we calculated and presented the monthly positivity rate for each pathogen. The positivity rate was defined as the number of positive cases divided by the number of tests having been carried out that month.

Results

Of the 1204 patients initially included in the COORDINATE trial, 30 (2.5%) were excluded due to lack of parental/guardian consent or inability to obtain a sample, resulting in a total of 1174 outpatient samples. A total of 802 of these samples were obtained from Chui between November 9 2022 and March 27 2023, and 372 from Naryn between November 1 and March 15. The 300 inpatient samples were collected between December 12 2022 and June 15 2023.

Inpatients were significantly younger than outpatients with larger proportions in the < 1 year and the 1–3 years groups. Significantly more in-patients were febrile, and more inpatients had an oxygen saturation below 93%. There was no significant difference in sex between the two groups (Table 1).

Additional symptoms and findings from inpatients are reported in Table 2. More in-depth characteristics of outpatients have been described elsewhere [14].

Microbiological findings

The frequencies of positive cultures, positive RT-PCRs, and rates of viral-bacterial simultaneous detection are reported in Table 3, and the frequencies of individual pathogens are reported in Table 4.

The most frequently identified bacterium in both in- and outpatients was *S. pneumoniae*, which was found in 62 (20.7%) inpatients and 119 (10.1%) outpatients, totally being detected in 181 out of 377 (48.0%) of patients with a positive culture, and accounting for 181 out of 404 (44.8%) of bacterial isolates. Of 39 beta-haemolytic streptococci identified in outpatients, 27 (69.2%) belonged to Lancefield group A. We identified no beta-haemolytic streptococci from inpatients. *M. pneumoniae* was identified in 47 (15.8%) of inpatients and 5 (0.7%) of outpatients (Table 4).

Table 3 Microbiological results from 300 inpatients and 1174 outpatients diagnosed with pneumonia and respiratory tract infection

Culture results	Inpatients, *N* = 300	Outpatients, *N* = 1,174
Positive culture	83 (27.7%)	294 (25.0%)
> 1 significant isolate	13 (4.3%)	12 (1.0%)
RT-PCR results	**Inpatients**, *N* = 296	**Outpatients**, *N* = 694
Positive, virus	162 (54.7%)	445 (64.1%)
Positive, atypical bacterial pathogen	48 (16.2%)	26 (3.7%)
>1 virus detected	36 (12.2%)	102 (14.7%)
Simultaneous detection*	56 (18.9%)	137 (19.7%)
No aetiology identified**	64 (21.6%)	177 (25.5%)

Samples were collected in two inpatient clinics in Bishkek and 14 outpatient clinics in the Naryn and Chui regions of Kyrgyzstan. Patients were aged 6 months to 12 years

*Positive RT-PCR for virus and bacterial growth OR positive RT-PCR for virus and positive RT-PCR for atypical bacterium

**RT-PCR and culture negative, of those with RT-PCR carried out

Abbreviations: RT-PCR, reverse transcriptase polymerase chain-reaction

Bacterial findings varied with age groups. In inpatients, *S. pneumoniae* was found with higher prevalence in the under-five age group when compared to the 5-12-year-olds ($p = 0.013$), whereas *M. pneumoniae* was more frequently observed in the 5-12-year-olds ($p < 0.001$). In outpatients, *S. aureus* ($p = 0.039$) was more frequently identified in the 5-12-year-olds, whereas *M. catarrhalis* ($p < 0.001$) was more frequently identified in the under-fives (Table 4).

Rhinovirus was the most frequently identified virus detected in 18.2% of inpatients and 24.6% of outpatients. In outpatients, RSV ($p = 0.011$) and bocavirus ($p = 0.009$) were more common in the under-fives, whereas influenza B ($p = 0.035$) and parainfluenza ($p = 0.025$) were more frequently observed in the 5-12-year-olds (Table 4).

Of 193 viral-bacterial simultaneous detections, *S. pneumoniae* was involved in 92 (47.7%).

Impact of PCV

Vaccination data were available for 278 (92.7%) of 300 inpatients and 1126 (95.9%) of 1174 outpatients. Significantly more outpatients than inpatients had received the age-appropriate PCV-doses (87.1% vs. 75.9%, $p < 0.001$,

Table 4 Pathogens from 300 inpatient children with pneumonia and 1174 outpatient children with respiratory tract infections, Kyrgyzstan

Culture results	Inpatients				Outpatients			
	Total ***N*** **= 300**	**< 5 y** ***N*** **= 219**	**5–12 y** ***N*** **= 81**	**p-value**[1]	**Total** ***N*** **= 1174**	**< 5 y** ***N*** **= 718**	**5–12 y** ***N*** **= 456**	**p-value**[1]
Streptococcus pneumoniae	62 (20.7%)	53 (24.2%)	9 (11.1%)	0.013	119 (10.1%)	79 (11.0%)	40 (8.8%)	0.217
Staphylococcus aureus	10 (3.3%)	7 (3.2%)	3 (3.7%)	0.733	34 (2.9%)	15 (2.1%)	19 (4.2%)	0.039
Haemophilus influenzae	9 (3.0%)	7 (3.2%)	2 (2.5%)	> 0.999	22 (1.9%)	10 (1.4%)	12 (2.6%)	0.127
Beta-haemolytic streptococci[2]	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	39 (3.3%)	18 (2.5%)	21 (4.6%)	0.051
Moraxella catarrhalis	13 (4.3%)	12 (5.5%)	1 (1.2%)	0.197	94 (8.0%)	76 (10.6%)	18 (3.9%)	< 0.001
Pseudomonas aeruginosa	2 (0.7%)	2 (0.9%)	0 (0.0%)	> 0.999	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
	Inpatients				**Outpatients**			
RT-PCR results	**Total** *N* = 296	**< 5 y** *N* = 217	**5–12 y** *N* = 79	**p-value**[1]	**Total** *N* = 694	**< 5 y** *N* = 436	**5–12 y** *N* = 258	**p-value**[1]
Influenza A	1 (0.3%)	1 (0.5%)	0 (0.0%)	> 0.999	51 (7.3%)	31 (7.1%)	20 (7.8%)	0.748
Influenza B	4 (1.4%)	2 (0.9%)	2 (2.5%)	0.290	42 (6.0%)	20 (4.6%)	22 (8.5%)	0.035
SARS CoV-2	17 (5.7%)	13 (6.0%)	4 (5.1%)	> 0.999	20 (2.9%)	11 (2.5%)	9 (3.5%)	0.459
Rhinovirus	54 (18.2%)	38 (18.0%)	16 (20.0%)	0.589	171 (24.6%)	110 (25.2%)	61 (23.6%)	0.639
Adenovirus	20 (6.8%)	18 (8.3%)	2 (2.5%)	0.081	66 (9.5%)	45 (10%)	21 (8.1%)	0.344
Metapneumovirus	41 (13.8%)	34 (15.7%)	7 (8.9%)	0.134	57 (8.2%)	39 (8.9%)	18 (7.0%)	0.361
Parainfluenza	41 (13.8%)	34 (15.7%)	7 (8.9%)	0.134	57 (8.2%)	28 (6.4%)	29 (11.2%)	0.025
RSV	9 (3.0%)	8 (3.7%)	1 (1.3%)	0.453	66 (9.5%)	51 (11.6%)	15 (5.8%)	0.011
Seasonal coronavirus	11 (3.7%)	7 (3.2%)	4 (5.1%)	0.492	25 (3.6%)	14 (3.2%)	11 (4.3%)	0.472
Bocavirus	5 (1.7%)	5 (2.3%)	0 (0%)	0.329	11 (1.6%)	11 (2.5%)	0 (0%)	0.009
Mycoplasma pneumoniae	47 (15.8%)	17 (7.8%)	30 (38.0%)	< 0.001	5 (0.7%)	4 (0.9%)	1 (0.4%)	0.656
Chlamydophila pneumoniae	1 (0.3%)	0 (0.0%)	1 (1.3%)	0.267	21 (3.0%)	14 (3.2%)	7 (2.7%)	0.715

Samples were collected in two inpatient clinics in Bishkek and 14 outpatient clinics in the Naryn and Chui regions of Kyrgyzstan. Patients were aged 6 months to 12 years. *Corynebacterium diphtheria* and *Legionella pneumophila* were not detected

[1]Chi-square test or Fisher's Exact Test as appropriate, based on differences between under-fives and 5–12 groups. *Italic*, $p < 0.05$

[2]27 were Lancefield group A, 7 were group G, 3 were group C, 1 was group B, 1 was not determined

Abbreviations: y, year; RT-PCR, reverse transcriptase polymerase chain-reaction

Table 1). In both outpatients and inpatients, 5-12-year-olds were more likely to have been vaccinated according to schedule than under-fives. Thus, in inpatients, 95.9% of 5-12-year-olds and 68.6% of under-fives ($p < 0.001$) and in outpatients, 97.5% of 5–12-year-olds and 80.3% of under-fives were vaccinated according to schedule ($p < 0.001$, Table 1).

Unvaccinated inpatients under five were more likely to have *S. pneumoniae* growth compared to their vaccinated counterparts ($p = 0.030$, Table 5). The effect could not be evaluated in 5-12-year-olds due to a low number of unvaccinated patients in this group. There was no significant difference in growth of *S. pneumoniae* between vaccinated and unvaccinated outpatients regardless of age.

The growth rates of all other pathogens were similar in PCV-vaccinated and -unvaccinated children.

Monthly variation

The monthly variation in pathogens identified by RT-PCR is shown in Fig. 1 for outpatients and Fig. 2 for inpatients.

Rhinovirus was frequent in both groups and did not have a positivity rate of under 10% in any study month. In outpatients, RSV showed a high positivity rate of 23.1% in November 2022, but the rate was 5.0% or lower in all other months. The SARS-CoV-2 positivity rate peaked in March 2023 at 15.8% in inpatients and 12.5% in outpatients. A small epidemic of influenza B in outpatients peaked in December 2022 with a positivity rate of 12.4%. Also in December 2022, Influenza A peaked in outpatients with a positivity rate of 27.3% after which it was scarcely or not detected.

Table 5 *S. pneumoniae growth* distributed by PCV-vaccination status

Inpatients	**Vaccinated *N* = 211 (75.9%)**		**Unvaccinated *N* = 67 (24.1%)**		
	SP growth	***No SP growth***	***SP growth***	***No SP growth***	**p-value**
Total	34	177	23	44	*0.002*[1]
<5 y	27	113	22	42	*0.030*[1]
5–12 y	7	64	1	2	0.294[2]
	Vaccinated *N* = 981 (87.1%)		**Unvaccinated** *N* = 145 (12.9%)		
Outpatients	*SP growth*	*No SP growth*	*SP growth*	*No SP growth*	p-value
Total	93	888	19	126	0.226[1]
<5 y	55	491	19	115	0.225[1]
5–12 y	38	397	0	11	0.610[2]

From 300 inpatients with pneumonia and 1174 outpatients with symptoms of respiratory tract infection in Kyrgyzstan. Patients were aged 6 months to 12 years

[1]Chi-square test. Italic, $p < 0.05$

[2]Fishers exact test. Due to small number of unvaccinated children in this group, the test was underpowered to show a difference

Abbreviations: Abbreviations: y, years; SP, *Streptococcus pneumonia*; PCV, pneumococcal conjugate vaccine

In inpatients, a consistent monthly rise of *M. pneumoniae* was seen from February 2023 (positivity rate 1.7%) to June 2023 (positivity rate 34.9%) possibly reflecting a local epidemic.

Discussion

To our knowledge this is the first set of published data on airway pathogens associated with RTI in children from Kyrgyzstan and Central Asia as a whole.

The data from our inpatients are consistent with The Pneumonia Etiology Research for CHILD Health (PERCH) study, reporting 61.4% of cases having viral aetiology and 27.3% having bacterial aetiology [7]. In the present study, we did not identify an aetiological agent in 21.6% of the inpatient samples for which RT-PCR and culture were done (Table 3), whereas the PERCH study identified an aetiological agent in over 98% of cases, possibly due to the elaborate setup.

The PERCH study further differed from ours in being multisite on two continents and including more specimen types (blood PCR and culture, urine antigen analysis, pleural fluid, etc.).

The distribution of aetiological agents and the timely variation of most viral agents in our study were generally comparable to what has been found in other studies of RTIs in children [6, 7, 16–21]. For example, rhinovirus displayed both an autumn peak and a spring peak in outpatients, a phenomenon that has been previously documented in long-term studies [20]. For *M. pneumoniae*, however, our study showed a surprisingly high frequency in inpatients. This was seen from March to June when as many as 34.9% of inpatients tested positive for *M. pneumoniae*. This suggests that a *M. pneumoniae* epidemic hit Kyrgyzstan during 2023, when it also caused epidemics elsewhere in the world due to lifting of pandemic restrictions [22, 23]. Surprisingly, *H. influenza* was found in only 2.6% of patients, which may indicate challenges in culturing the pathogen in our laboratory or could be due to a high vaccination rate [12, 24].

Our study confirms that RSV is more common in younger children, whereas influenza is more common in older children [6, 16, 19], though in our study it was only the case for influenza B. We also found that *S. pneumoniae* was more common in inpatient under-fives, though this could be due to a higher rate of unvaccinated children in this age group (Table 5).

Co-infections contribute significantly to morbidity in children with RTI [3, 8, 25], and especially viral infections with co-occurrence of *S. pneumoniae* have been of special interest in the literature, as summarized in a recent review [6]. However, the direction of the complex dynamic between viral infections and different *S. pneumoniae* serotypes has not been clearly established. In our study, we observed that *S. pneumoniae* was not

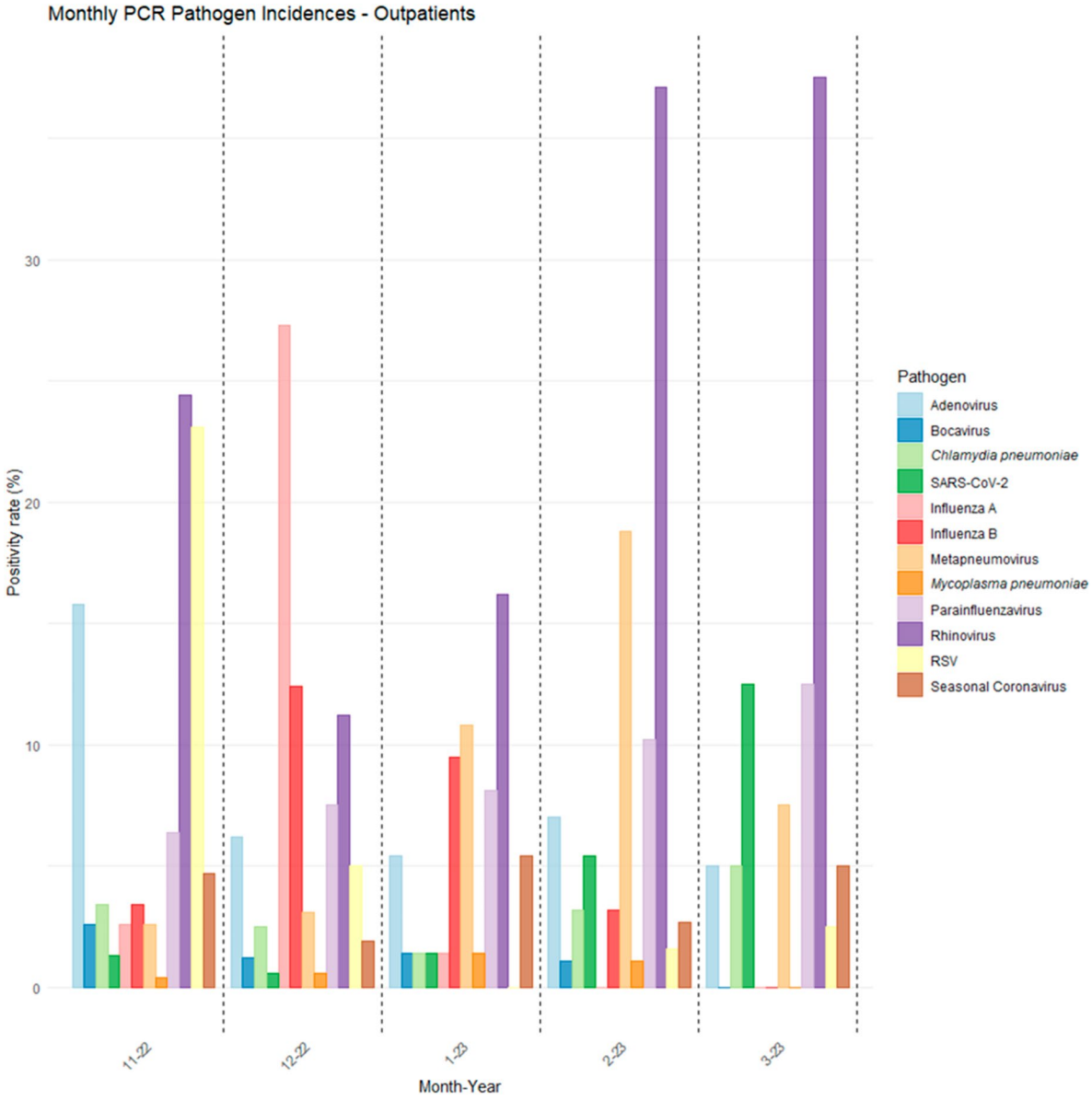

Fig. 1 Positivity rate of 694 RT-PCR analyses of Kyrgyz outpatient children with symptoms of respiratory tract infection. Samples were collected between November 2022 and March 2023 in the Naryn and Chui regions of Kyrgyzstan. Samples were also PCR tested for Legionella pneumophila, but this was not identified. Abbreviations: RT-PCR, reverse transcriptase polymerase chain-reaction; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2

overrepresented in viral-bacterial simultaneous detections, as it was found in 48.0% of those with a positive culture and in 47.7% of viral-bacterial simultaneous detections.

We observed a slightly lower PCV coverage rate of 87.1% in outpatients compared to the official coverage of 92%, as reported by the WHO/UNICEF estimates of National Immunization Coverage. However, the coverage rate of the 5-12-year-old outpatients was 97.5%. This difference could be due to delays in vaccination for the smaller children or due to external factors such as the COVID-19 lockdown [26].

It is well-established that PCV protects against invasive pneumococcal disease [10] and consequently reduces the risk of hospital admission. Our finding that inpatients were more likely to be unvaccinated for *S. pneumoniae* (Table 1) suggests a protective effect of the vaccine against the risk of hospitalisation due to pneumococcal pneumonia.

Vaccination programs affect the carriage rate of *S. pneumoniae* and change the predominant serotypes associated with both disease and colonisation [11, 27–30]. Future studies focused on identifying the serotypes of *S.*

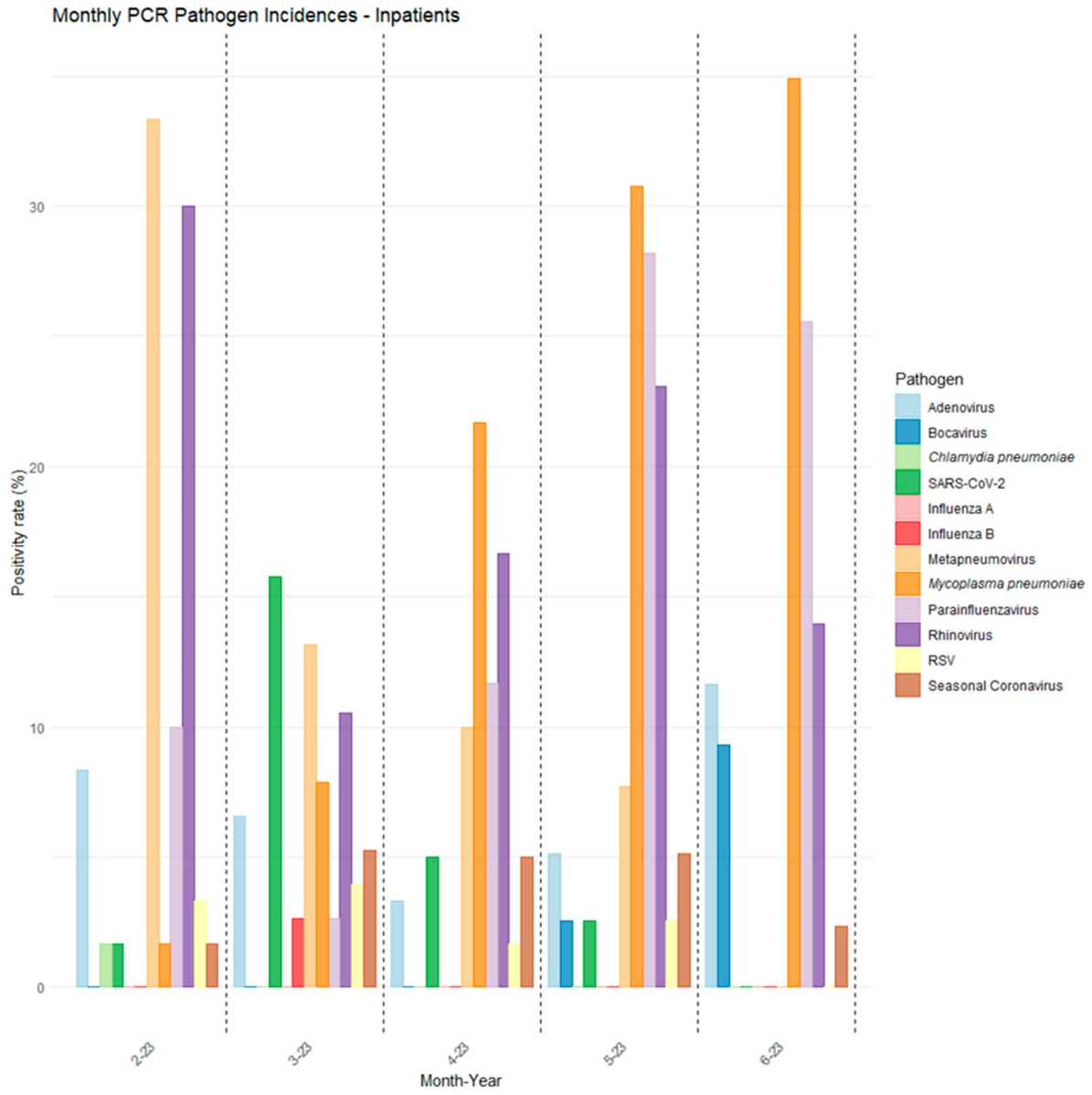

Fig. 2 Positivity rate of 296 RT-PCR analyses of Kyrgyz inpatient children with a diagnosis of pneumonia. Samples were collected between February 2023 and June 2023 in two inpatient clinics of the Kyrgyz capital of Bishkek; National Centre of Maternity and Childhood Care (NCMCC) or City Children's Clinical Hospital. Samples were also PCR tested for *Legionella pneumophila* but this was not identified. Due to a low number of included patients, December 2022 and January 2023 are not included in this plot. See appendix 1. Abbreviations: RT-PCR, reverse transcriptase polymerase chain-reaction; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2

pneumoniae in Kyrgyz children with RTI could provide valuable insights into vaccine-serotype dynamics.

Strengths and limitations

A major strength of this study was the collection of samples from both inpatients and outpatients over several months during the RTI season, using both culture and multiplex PCR methods which enabled us to comprehensively identify both bacterial and viral agents, as well as assess the rate of co-infections in respiratory infections in Kyrgyz children.

The broad age range of 6 months to 12 years included in this study was wider than in most previous studies often focusing on under-fives [6]. We have thus provided a comprehensive and detailed picture of airway pathogens in Kyrgyz children.

A major limitation of our study was that we mainly used upper airway sampling for bacterial culture. Upper airway cultures may largely represent colonisation rather than actual aetiological pathogens, especially in the case of *M. catarrhalis* [6, 7], and ultimately, this could mean that the true rates of bacterial infections are lower than

reported in our study and underlines that not all viral-bacterial simultaneous detections in our study necessarily represented actual co-infections. We tried to mitigate this limitation by demanding a higher rate of growth (two streak zones) for pathogens more likely to be colonisation (*M. catarrhalis*, *S. aureus*). However, many positive cultures—including *S. pneumoniae*—may still be colonising rather than aetiological agents. Nevertheless, the observation that inpatient samples collected by suction were more frequently positive on both RT-PCR and culture (data not shown) suggests that this limitation may to a lesser extent apply to inpatients.

Another limitation of our study was the timely distribution of the sampling, especially considering that inpatient and outpatient samples were collected in different, though overlapping periods, limiting comparability between the two groups. For example, while we found RSV to be more common in outpatients, this could simply reflect that we did not have inpatient samples from the month with most RSV cases (November 2022). The opposite was the case for *M. pneumoniae*, where the epidemic in inpatients could not be found in outpatients. In conclusion, our findings should not be used to compare aetiologies of inpatients and outpatients in general. Furthermore, collecting samples over one year would give a much better view of the epidemiological properties of various pathogens and would make the study more easily comparable to the literature [7].

Lastly, including inpatients based on a primary diagnosis of pneumonia may have posed a limitation. This was chosen pragmatically to facilitate patient inclusion as we did not expect a significant number of children with upper RTI to be admitted as inpatients. Still, it was a less specific inclusion criterion compared to the outpatients, who had to exhibit certain symptoms within a specific time frame. While all 300 included inpatients had cough, 55 (data not shown) did not present with chest indrawing or fast/difficult breathing, which is part of the criteria for a diagnosis of pneumonia according to the Integrated Management of Childhood Care (IMCI) guidelines from the World Health Organization [31]. However, 97.3% of inpatients had chest X-rays showing consolidation, a strong paraclinical indicator of pneumonia (Table 2).

Conclusion

In our study of children aged 6 months to 12 years with RTI in Kyrgyzstan, we found that viral infections accounted for more than half of the cases, far exceeding bacterial infections. One in five patients had a simultaneous detection of a virus and a bacterium. The most common viral agent in our study was rhinovirus, found in almost one in four cases, while *S. pneumoniae* was the most common bacterial pathogen. *S. pneumoniae* was particularly abundant in young and unvaccinated inpatients, and PCV coverage rates were lower than expected, especially in inpatients under five. This study provides valuable baseline data for new guidelines and further research on airway pathogens in Central Asia.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

JB, JALK, JKj, TS, AP, RS, ACYN, CSJ and EI and NSK conceptualised the project. JB, EI and NO planned the data collection and the database. JB wrote standard operating procedures (SOPs). NO, AE, MM and AA and AT translated the SOPs and were in charge of logistics during data collection including coordinating the work of study assistants, drivers, data entry assistants etc. RT and MZ were in charge of planning and carrying out analyses of bacteriological samples. EB and GE were in charge of planning and carrying out PCR analyses. CSJ, ACYN, NSK and JALK stood for supervision of data collection and analysis of bacteriological samples during the course of the data collection. TS stood for supervision of the local team. ARB made firsts drafts of figures, tables and parts of the manuscript.NO and JB analysed the data and wrote the final drafts of the manuscript. The two first authors NO and JB contributed equally to the manuscript. The three last authors JKj, TS and JALK contributed equally to the manuscript.All authors revised and provided comments to the manuscript. All authors agreed on the final version.

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Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. Frequencies of co-findings of individual pathogens are available in Appendix 1. Data for Figs. 1 and 2 is available in Appendix 2.

Declarations

Ethics approval and consent to participate

The study was approved on 18 June 2021 by the Ethics Committee (ref: no. 1) of the National Centre of Maternity and Childhood Care, Bishkek. A data processing agreement was prepared and approved by all stakeholders. Parents or guardians of all study participants had to give informed written and oral consent prior to inclusion. The study was conducted in accordance with the Declaration of Helsinki [32].

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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